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Detection of frequency modulation in the FM-bat *Phyllostomus discolor*

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Abstract In a two-alternative forced-choice procedure lesser spear-nosed bats, *Phyllostomus discolor*, had to discriminate between a pure tone stimulus and a sinusoidally frequency-modulated signal generated at the same carrier frequency as the tone. Modulation depths of the SFM stimuli were reduced until the animals' performance dropped below the 75%-correct level which was used to determine difference limens for detection of frequency modulation (FMDL). The dependence of FMDLs on modulation and carrier frequency was systematically investigated. For a carrier frequency of 18.5 kHz, average FMDLs increased from 95 Hz at a modulation frequency of 10 Hz to 820 Hz at a modulation frequency of 2000 Hz which corresponds to Weber ratios ($2\Delta f/f$) of 0.005 and 0.044 respectively. Further, difference limens were found to increase linearly in proportion to carrier frequency throughout a major part (9–74 kHz) of the species' hearing range. In comparison to other mammals, *P. discolor* has a pronounced capability for frequency discrimination which might be related to the extensive use of individually distinct frequency-modulated communication calls and audio-vocal learning.

Key words Frequency discrimination · Hearing · Psychoacoustics · Operant conditioning · Chiroptera

Abbreviations FM frequency modulation · SFM sinusoidal frequency modulation · FMDL frequency modulation difference limen · CF constant frequency · DLF difference limen for frequency · VCO voltage-controlled oscillator · SPL sound pressure level · WR Weber ratio

Introduction

Bats of the suborder Microchiroptera are well-known for their ability to use echolocation for short distance orientation, obstacle avoidance and, depending on the species, for catching prey. They are commonly subdivided into CF-FM and FM-bats according to the type of echolocation call. The orientation calls of CF-FM-bats are dominated by a long constant frequency (= CF) component preceding the call's frequency-modulated (= FM) end. In contrast, species uttering frequency-modulated echolocation signals containing FM components only, like *Phyllostomus discolor*, are referred to as FM-bats (for details see Neuweiler and Fenton 1988).

Beside echolocation calls, some species of bats possess a complex repertoire of social calls (Fenton 1985). In the lesser spear-nosed bat *P. discolor*, calls uttered in the context of mother-infant communication are important and have been analyzed structurally and behaviorally (Esser and Schmidt 1989, 1990; Esser 1994). The maternal "directive calls" are characterized by an individual type of sinusoidal frequency modulation (= SFM) pattern (Esser and Schmidt 1989). Beside modulation frequency, modulation depth and number of modulation cycles per call contribute to the mother's vocal signature. Infant bats, in the course of ontogeny, adapt their isolation calls to the corresponding call characteristics of the own mother (Esser and Schmidt 1989) and even to a computer-generated directive call, if hand-reared in the absence of conspecifics (Esser 1994).

Despite their marked individuality, maternal directive calls of this species can be regarded as highly determined signals because modulation frequencies seem restricted to a small band ranging from 76 to 100 Hz. Since in different mammals (including man and bats) the ability to detect sinusoidal frequency modulations is substantially influenced by the modulation

frequency (Zwicker and Fastl 1990; Bartsch and Schmidt 1993), the question arises, whether or not there is a correlation between the sensory acuity of *P. discolor* and the limited range of modulation frequencies used in the context of intraspecific acoustic communication. Beside the modulation frequency the modulation depth of calls is also influenced through audio-vocal learning (Esser 1994). Since the frequency discrimination ability of *P. discolor* has not yet been determined, a further goal of the present study was to provide the data for a frequency-based comparison of experimentally measured thresholds (present study) and interindividual differences in call structure (Esser and Schmidt 1989).

Two methods of measuring frequency discrimination ability have been commonly used in mammals (Moore 1993). The difference limen for frequency (DLF) is determined in discrimination experiments with successively presented steady tones slightly differing in frequency. In contrast, measuring frequency modulation difference limens (FMDL) involves tones that are frequency-modulated, typically at low rates. Here the amount of modulation depth required for detection of the modulation (with reference to an unmodulated pure tone) is determined.

In the present study the determination of frequency modulation difference limens (FMDL) was favoured for several reasons. First, the question concerning the significance of the limited range of modulation frequencies found in the species' directive calls could be answered within the same experimental paradigm (SFM stimulus vs. pure tone). Second, communication calls of *P. discolor* are SFM signals. Thus, the use of similar acoustic stimuli in a behavioral experiment should enable the bats to perform more rapidly and/or consistently. Third, behavioral data on the perception of SFM stimuli are available for only a single species of FM-bat, *Tadarida brasiliensis* (Bartsch and Schmidt 1993). Thus, interspecific comparisons of FMDLs are not yet possible within this group of bats. Further, such a comparison should demonstrate whether or not in *P. discolor* the dependence on SFM communication calls (Esser and Schmidt 1989; Esser and Daucher 1996) is even reflected in low absolute thresholds of this species. Since FMDLs not only depend on modulation frequency but also on stimulus duration (for details see discussion), both stimulus parameters were adopted from the study of Bartsch and Schmidt (1992) to make results comparable. In *T. brasiliensis*, FMDLs were determined at a carrier frequency of 40 kHz, close to the center frequency of the fundamental of echolocation calls (Bartsch and Schmidt 1993). For most species studied (Fay 1974), discrimination thresholds obtained at different reference frequencies, at least for frequencies above 2 kHz, can be compared by Weber ratios (DLF: $\Delta f/f$, Keidel 1975; FMDL: $2\Delta f/f$, Zwicker and Fastl 1990). Thus, further characteristics of acoustic stimuli used in the present study were designed accord-

ing to species-specific calls (Esser and Schmidt 1989). The carrier frequency of the SFM stimuli was set at 18.5 kHz, which in *P. discolor* corresponds to the average carrier frequency of the directives' fundamental. Accordingly, at the beginning of our experiments, the modulation depth was set to 8 kHz, which equals the mean value of modulation depth ($2\Delta f$) of the directive calls' fundamental (Esser and Schmidt 1989).

In order to reinforce the expected natural preference for SFM stimuli, FMDLs of *P. discolor* were determined in a two-alternative (SFM stimulus vs. pure tone), forced-choice procedure, providing a food-reward upon each discrimination of a SFM signal. In addition to the SFM signals described above, different carrier frequencies were tested at a fixed modulation frequency in order to obtain Weber ratios ($2\Delta f/f$) throughout the major part of the species' hearing range (Esser and Daucher 1996).

Materials and methods

Four adult male *Phyllostomus discolor*, caught in the province of Guanacaste in Costa Rica (85° 10' W; 10° 30' N), were used for the experiments after an acclimatization period in the laboratory of two months. Animals were housed together except during individual experimental sessions. In their cage (1.1 × 0.6 × 0.6 m), the animals had free access to water and were exposed to a LD-cycle of 12:12 h with dimmed red light during the light phase. Ambient temperature and relative humidity were maintained at 25°C and ≥ 70% respectively. During the period of behavioral tests, the bats received exclusively meal worms (larvae of *Tenebrio molitor*) as food reward. Once a week, the meal worms were dusted with vitamins (Merck, Combionta) and minerals (Vita, Vitakalk) prior to feeding. With an average of thirty meal worms consumed per bat per day during the tests, the animals' daily controlled body weight was kept within the normal range (33–45 g; Emmons and Feer 1990).

For behavioral training and threshold determination the bats were transferred each day to a vertically mounted, inverted Y-maze within a sound-proof chamber at the beginning of the dark period. The wooden maze (see companion paper by Esser and Daucher, Fig. 1) consisted of a start cage (0.2 × 0.25 × 0.3 m), a decision chamber with a trapezium-shaped base of 0.075 m² and two 0.8 m long alleys. The inside walls of the maze were covered by convoluted, anechoic polyurethane foam whereas the back and the front consisted of carpet and acrylic glass respectively. In order to further minimize the probability of multiple acoustic reflections, the walls of the alleys were arranged slightly nonparallel. The start cage could be opened and closed at the beginning and end of each trial by a horizontal slide. A high-frequency loudspeaker (Technics, TH 800 B) was mounted at the end of each alley, and a small reward dish was positioned near each loudspeaker. Single mealworms could be supplied to the dishes from outside the maze by a pair of funnels with attached teflon tubes.

For the generation of the acoustic stimuli, the following system was used. The pure tone reference signals were generated by a function generator (Voltcraft, FG 1617). For the synthesis of the SFM stimuli a voltage-controlled oscillator (Exact, Model 129) was used in combination with a computer-based (PC 386) D/A-converter system (AZNB, Tacita). This system consisted of the D/A converter running at a conversion speed of 250 kHz with an amplitude resolution of 12 bit, an anti-aliasing filter and a computer-controlled attenuator. The output of the system was connected to the external FM input of the voltage-controlled oscillator (VCO), thus controlling modulation frequency and depth of the SFM stimuli. Both the

carrier frequency of the SFM signal and the frequency of the tone stimulus were checked regularly using a frequency counter (Thandar, TF 1000). For envelope shaping and generation of a stimulus repetition rate of 1 Hz, a custom-made electronic switch was used on both channels. The switches were triggered alternatively thus preventing temporal overlap of both stimuli. Further, a bandpass filter (Kemo, VBF/8; 90 dB/octave) with the cutoff frequencies centered at the used pure tone frequencies was introduced to eliminate possible faint harmonics. On the SFM channel, harmonics were at least -40 dB below the signal and could not be detected (Nicolet, 446B FFT Computing Spectrum Analyzer). The generated 500 ms pulses (including 50 ms rise and fall time) were fed into a pair of attenuators (Hewlett-Packard, 350 D) and the timing and amplitude checked with an oscilloscope (Gould, DSO 1602). Subsequently, the generated signals were amplified by two separate amplifiers (Denon, PMA 1060). Finally, the signals were transduced alternately via the high frequency speakers. The sound pressure level (SPL) of both signals was adjusted to 80 dB SPL (rms), measured in the symmetry axis of the Y-maze at the lower margin of the start cage, and regularly checked using a gridless condenser microphone (Brüel and Kjaer, 4135), a preamplifier (B & K, 2633) and a measuring amplifier (B & K, 2610). Both types of stimuli could be switched manually to the left or the right alley, thus making a pseudo random scheme of stimulus presentations possible.

The calibration of the settings of the computer-controlled attenuator into frequency values describing the modulation depths ($2\Delta f$) of SFM signals was performed for all attenuation steps, modulation and carrier frequencies by high-resolution zero-crossing analysis. Since the FMDLs determined in the present study depend on this procedure it is described briefly below.

Due to the time-bandwidth product limitation of Fourier transforms we were not able to calibrate the transduced SFM stimuli by conventional high-resolution FFT-analysis (e.g. Engineering Design, Signal) with a performance comparable to the sensory acuity of the bats. In zero-crossing analysis the instantaneous frequency (f) of a time-varying waveform is measured directly from the elapsed time (t) between successive zero-crossings of the original time signal ($f = 1/(2t)$). Thus, it provides a substantially higher spectral-time resolution than Fourier-based algorithms. However, due to the absence of time-averaging in zero-crossing analysis, this method becomes highly vulnerable to the presence of even weak harmonics or faint noise and thus performs poorly on most acoustic signals. However, due to the high signal-to-noise ratio and due to the substantial exclusion of harmonic distortions, the stimuli generated as described in the present study were highly suited for calibration by this procedure.

For calibration, all stimuli were generated and transduced via the loudspeakers as described above and subsequently recorded, analogous to the SPL measurements, with the Brüel & Kjaer equipment. The output of the measuring amplifier (B & K, 2610) was serially connected to a 100 kHz low pass filter (Kemo, VFB/8; 90 dB/octave) and a computer-based (PC 386) sound analysis system (Engineering Design, Signal). Via the A/D-converter of the system (Data Translation, DT2821-G-8DI; sample rate: 250 kHz; amplitude resolution: 12 bit) and direct memory access, the digitized samples were transferred into the computer memory for software-based (Engineering Design, Signal) zero-crossing analysis. Subsequently, time segments of 300 ms, one for each stimulus configuration (modulation frequency, modulation depth, carrier frequency), were extracted from these recordings. The zero crossings within these time segments were detected and each resulting data set was scaled into a histogram (frequency vs. counts/bin) with an absolute bin width of 10 Hz (examples in Fig. 1). From these spectrograms, measurements of modulation depth were taken at an amplitude level of 50% re full scale (dashed lines in Fig. 1). Further, in order to avoid a disproportion between the A/D-conversion rate and the frequency of the sampled time signal, all stimuli with a carrier frequency of 74 kHz were recorded on a high-speed tape recorder (Racal, V-Store; recording speed: 30 in/s) prior to being fed into the computer system at 0.5-fold reduced speed. A corresponding correction factor was used

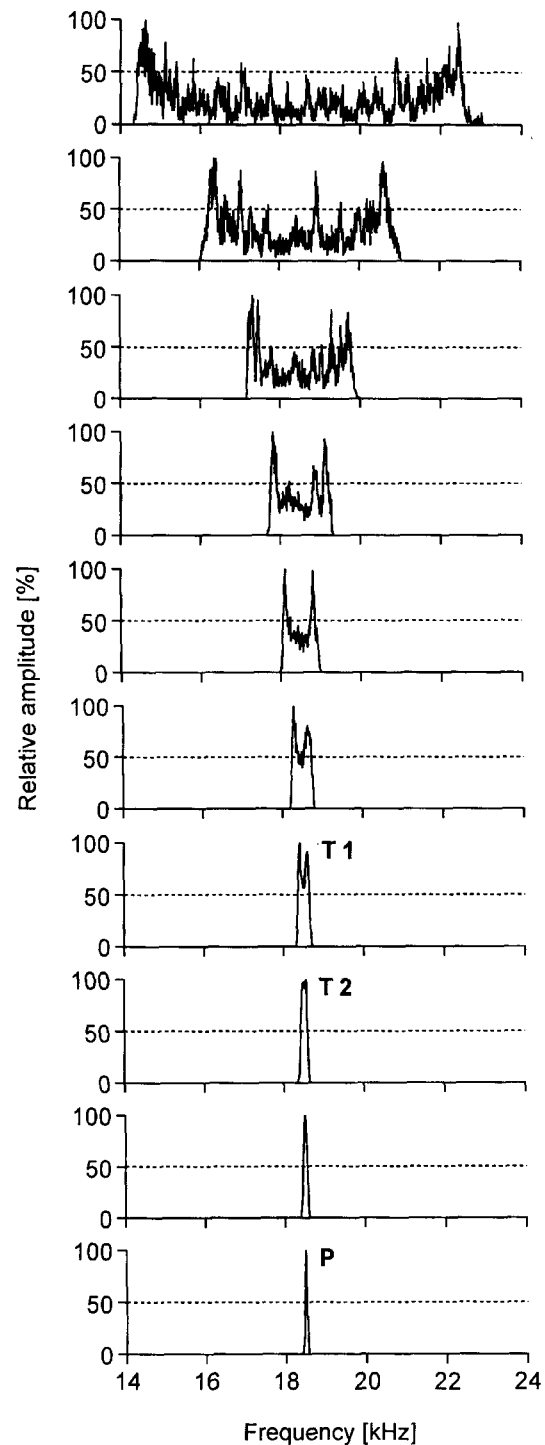


Fig. 1 Histograms (frequency vs. counts/bin = rel. amplitude) resulting from zero-crossing analyses of 300 ms segments of SFM stimuli (carrier frequency: 18.5 kHz; modulation frequency: 100 Hz). The succession of spectrograms (top to bottom) corresponds to lowering the voltage controlling the VCO stepwise by 5 dB. A corresponding analysis of the pure tone stimulus (*P*) is shown for comparison. *T1* and *T2* indicate threshold modulation depths ($2\Delta f$) for bats 1 (180 Hz) and 2 (300 Hz). Additional explanations in the text (Materials and methods)

later to compensate for the resulting difference in relative sampling speed.

Experimentally, the determination of the bats' FMDLs proceeded as follows. After the naive animals had been familiarized with the Y-maze, single meal worms were presented when a bat reached the reward dish in the alley where the speaker transmitted the SFM signal (carrier frequency: 18.5 kHz; modulation depth: 8 kHz). During this initial training period, a pure tone reference was not presented. After receiving the reward, the bat was encouraged to reenter the start cage by aversive noise or by gently pushing it. Bats that had made a wrong choice were allowed to correct it by entering the opposite alley. In a later stage of the experiments, only the first choice of a bat, if correct, was rewarded. Bats choosing the wrong side first had to reenter the start chamber voluntarily for the next trial without receiving a reward. From this time onwards, signals were presented on both sides, with one speaker transducing the pure tone reference of 18.5 kHz. In order to cope with various strategies of the bats, which were similar to those described in the companion paper by Esser and Daucher (pp. 779–785), the sequences of stimulus positions (left or right) were chosen subjectively by the experimenter until the strategies disappeared and a stable level of correct choices (>75%) had been established for a particular bat. For the subsequent threshold determination all l/r-sequences were generated pseudorandomly following binomial statistics, but excluding repetitive sequences >3. A similar procedure has been described by Neuweiler (1962).

Prior to testing of SFM stimuli with carrier frequencies of 9, 37 and 74 kHz, together with the corresponding pure tones, complete threshold data were collected for SFM signals with a carrier frequency of 18.5 kHz. Generally, only one modulation depth, one modulation frequency, and one carrier frequency per bat was tested daily. During the first ten trials at the beginning of each session, only signals with the maximum modulation depth for a particular carrier frequency were used on the SFM channel. If a bat responded positively in at least eight of these ten trials, the modulation depth was subsequently reduced by lowering the voltage controlling the VCO by 5 dB. Sessions, where bats made less than eight correct choices within these first ten trials, were continued without reducing the modulation depth and thus were not used for threshold determination. All trials with stimuli of maximum modulation depth were summed for a particular day and bat and the percentage of correct choices within these trials was referred to as daily performance (see Results). Reduction of the modulation depth in 5 dB steps of the attenuator controlling the VCO continued until the bat's performance dropped below the previously defined 75% level for a particular stimulus configuration (carrier frequency, modulation frequency and modulation depth). The threshold determined this way was retested in a second series, typically starting next day. If, in this second series, the threshold modulation depth ($2\Delta f$) differed from the previous one, the entire procedure continued until a reproducible threshold was found. The lowest modulation depth eliciting a positive response was regarded as the FMDL for a particular carrier, modulation frequency and bat.

According to this procedure, frequency modulation difference limens were determined for eight modulation frequencies (10, 20, 50, 100, 200, 500, 1000, 2000 Hz) and four carrier frequencies (9, 18.5, 37, 74 kHz).

Results

Daily performances

The daily performance as defined above is shown for all bats in Fig. 2. Bats 1, 2 and 3 learned the basic task of discriminating between the SFM signal with a carrier of 18.5 kHz and a modulation depth of 8 kHz and the

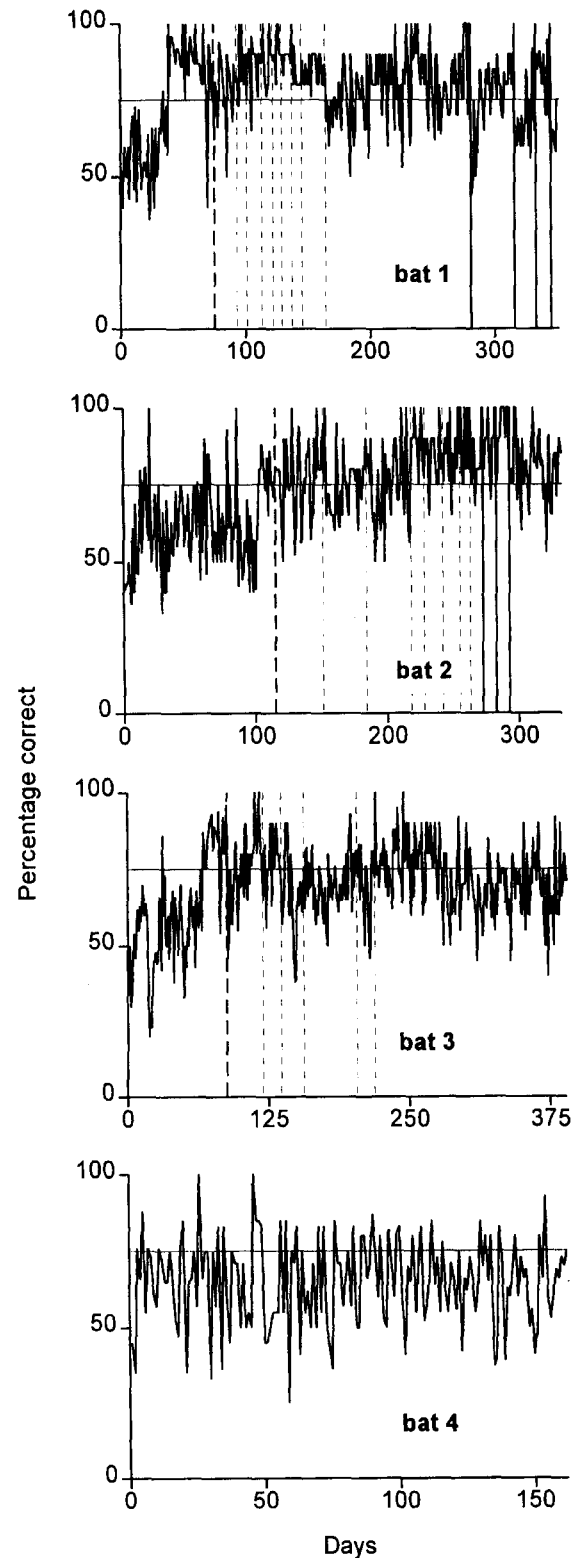


Fig. 2 Daily performances of 4 bats. The 75%-correct level is indicated by the horizontal line in each graph. Vertical lines indicate the first reduction of modulation depth (**bold dashed line**), changes in modulation frequency (*dashed*) and changes in carrier frequency (*continuous*) of the SFM stimuli. Note different time scales

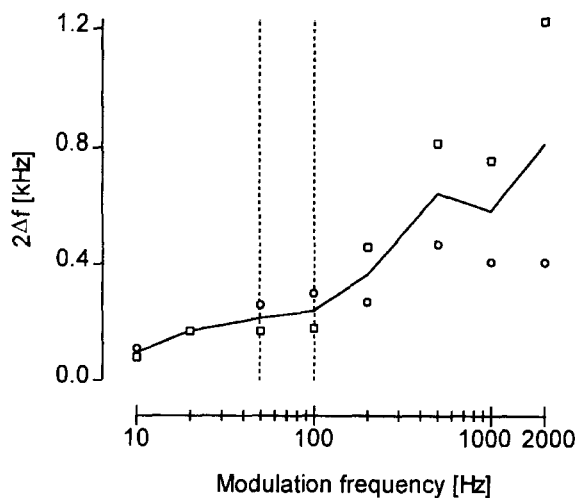


Fig. 3 FMDLs ($2\Delta f$) obtained from bat 1 (squares) and bat 2 (circles) at 8 different modulation frequencies (carrier frequency = 18.5 kHz). The corresponding means are indicated by the continuous line. The interval between the dashed lines specifies the range of modulation frequencies (49–100 Hz) covered by infant isolation calls and maternal directive calls of *P. discolor* (calculated from data of Esser and Schmidt 1989)

corresponding pure tone reference within about 40 (bat 1), 70 (bat 3) and 110 days (bat 2). In contrast, bat 4 never reached a good performance in this frequency discrimination task whereas bats 1 and 2 showed a consistent level of correct choices throughout the rest of the study period. However, even in these well-performing bats the percentage correct for the trials with stimuli of maximum modulation depth repeatedly dropped below the 75% level (horizontal lines in Fig. 2). Often these decreases could be correlated with changes in modulation or carrier frequency whereas in other cases no explanation for this behavior could be found.

Frequency modulation difference limens

At a carrier frequency of 18.5 kHz, complete threshold data for 8 modulation frequencies could be obtained from two animals (bats 1 and 2). Although the discrimination ability of bat 3, at the first tested modulation frequency (= 100 Hz), was comparable to the performances of bats 1 and 2, it was unable to adjust to changes in modulation frequency even over a prolonged period of time (Fig. 2). Thus, the threshold data in Fig. 3 are restricted to bats 1 and 2.

In both animals, lowest $2\Delta f$ values of 80 and 110 Hz respectively were measured at the lowest tested modulation frequency (= 10 Hz; Fig. 3). Highest $2\Delta f$ values were determined when stimuli with modulation frequencies ≥ 500 Hz were presented. In bat 1, a remarkable threshold increase could be observed up to a modulation frequency of 2000 Hz. The thresholds determined in bat 2, tend to form a plateau in the

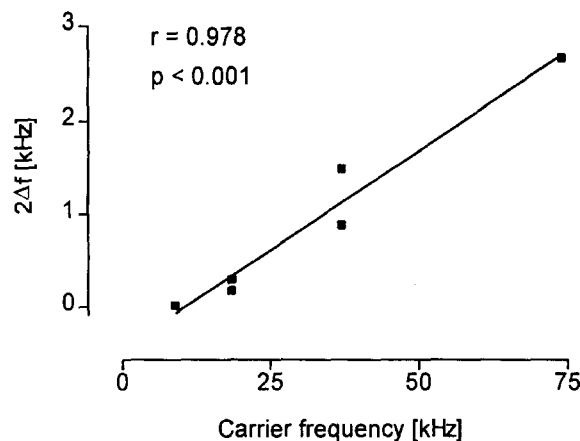


Fig. 4 FMDLs ($2\Delta f$) as a function of carrier frequency for a modulation frequency of 100 Hz. Pooled data from bats 1 and 2 and computed regression line (linear regression: $r =$ correlation coefficient). Additional explanations in the text (Results)

corresponding range of modulation frequencies (≥ 500 Hz) or even show a minor tendency to decline. The average threshold curve shows a clear correlation between increasing modulation frequency and increasing $2\Delta f$ values. FMDLs in the range of the small band of modulation frequencies covered by species-specific calls (dashed lines in Fig. 3) show no obvious peculiarities.

After testing eight modulation frequencies at a carrier frequency of 18.5 kHz, SFM signals with a fixed modulation frequency of 100 Hz were presented at different carrier frequencies (9, 37 and 74 kHz). The data of bats 1 and 2 are shown in Fig. 4, including the corresponding thresholds from the previous experiment (modulation frequency: 100 Hz; carrier frequency: 18.5 kHz). In bat 2, thresholds could be obtained at each tested carrier frequency. In contrast, bat 1 repeatedly refused to cooperate when stimuli with carrier frequencies of 9 and 74 kHz were presented. Despite the limited number of FMDLs ($n = 6$) a highly significant linear correlation between the carrier frequency and the determined $2\Delta f$ -values was found (regression line in Fig. 4; correlation coefficient: $r = 0.978$) with the slope differing significantly from zero ($p < 0.001$; F-test). As shown in Fig. 4, FMDLs ($2\Delta f$) increase linearly by approx. 43 Hz per kHz increase in carrier frequency (f). Thus, the ratio of $2\Delta f/f$ seems to be constant at least over the corresponding part of the animals' hearing range.

Amplitude modulation test

As in the present study, the generation of frequency-modulated acoustic stimuli includes their transmission by a chain of interconnected devices and their final transmission via a loudspeaker. It is commonly

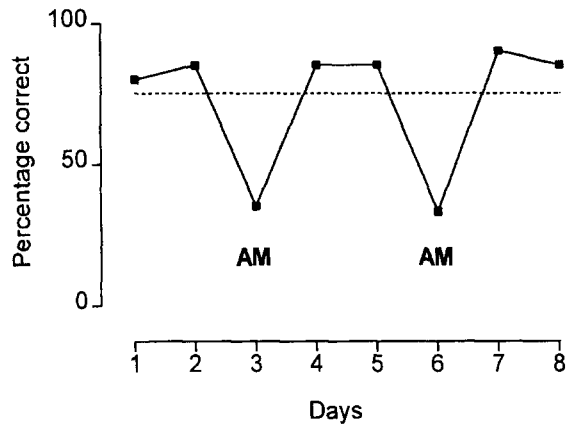


Fig. 5 Percentage of correct responses of bat 2 during the amplitude modulation test. The 75%-correct level is indicated by the horizontal line. AM = two sessions where amplitude-modulated stimuli were presented instead of FM signals

accepted that, due to the frequency responses of individual system components, frequency modulations may be superimposed by amplitude modulations of the same modulation frequency which can not be excluded. Thus, substituting the SFM stimulus with a sinusoidally amplitude-modulated (= SAM) signal with a carrier frequency equal to the one of the pure tone reference should resolve whether or not the bats might have used amplitude cues in the previous frequency discrimination task. Therefore, an experimental series was interspersed with days where SAM-signals with an amplitude modulation depth of 6 dB and a modulation frequency of 100 Hz were presented instead of the SFM signals (procedure modified according to Bartsch and Schmidt 1993). As shown in Fig. 5, in the absence of spectral cues deriving from SFM stimuli, the bat did not simply perform close to chance, but instead, demonstrated what appeared to be an active avoidance response (percentage correct: 35% (day 3); 33% (day 6)). Thus, we are confident that the decision of the bats to respond to SFM signals was not influenced by the possible availability of additional amplitude cues.

Discussion

Conditioning and test paradigm

Using a two-alternative forced-choice procedure and a modified method of limits (Penner 1995) we determined frequency modulation difference limens in *Phyllostomus discolor*. Comparing the training effort and outcome in the present study to the performances of bats reported in the companion paper (Esser and Daucher 1996), frequency discrimination is far more difficult for bats to learn than the detection of a pure tone stimulus. This is in accordance with findings in

other mammals as recently reviewed by Long (1994). In the present study, three characteristics of the acoustic stimuli were changed systematically, the modulation depth, the modulation frequency and the carrier frequency. Thus, the animals had to cope with the situation of a frequently changing rewarded stimulus. In this context the bats might have learned to avoid the unrewarded reference signal which was predictable in structure except for the few changes in carrier frequency. However, a part of the decline in daily performance (Fig. 2) is correlated with changes in modulation frequency, a situation where the pure tone stimulus remained exactly the same. Since other stimulus changes were not accompanied by a significant decrease in behavioral performance, it is not possible to decide on which basis the animals might have changed their performance level. Since three of the four animals tested learned the basic task to discriminate between a SFM signal mimicking a species-specific call and a corresponding pure tone reference, the present behavioral approach also seems suited for further psychoacoustic studies in this species. The same applies to the training apparatus and the feeding scheme which allowed up to 389 consecutive days of training and testing without any obvious deficits in the animals' state of health.

Difference limens

DLFs or FMDLs for a stimulus or carrier frequency around 18.5 kHz are available for a number of mammalian species or can be calculated from Weber ratios when determined at other frequencies. The latter, however, presupposes that the ratios of $\Delta f/f$ or $2\Delta f/f$ are constant throughout the hearing range of a particular species. In fact, at least above frequencies of 1 or 2 kHz, frequency difference limens change linearly as a function of frequency for nearly all animals studied so far (Fay 1974, 1988). It should be considered, however, that the comparability of data is limited by the following facts. Particularly at lower stimulus frequencies, different thresholds may be obtained with the DLF and the FMDL method (Moore 1993) whereas these differences tend to decline at higher frequencies of a species' hearing range (Long 1994). As confirmed in the present study, FMDLs are strongly influenced by the modulation frequency. Thus, interspecies comparisons should be standardized by using FMDLs obtained at the ideal modulation frequency for a particular species and test paradigm. Further, difference limens are influenced by sensation level and stimulus duration. However, according to Fay's extensive survey of literature (Fay 1988), both correlations may be neglected at higher sensation levels above about 50 dB SPL and at sound durations longer than about 400 ms. Accordingly, a sensation level and a stimulus duration as used in the present study (55 dB SPL; 500 ms) seem beyond the ranges of significant threshold variations.

Table 1 Comparison of FMDLs and DLFs obtained from several echolocating and non-echolocating mammals including man

Species	Author	Frequency [kHz]	SPL	Δf [Hz]	WR	Method
Man	Zwicker and Fastl 1990	18.5 ^a	60 phon	129 ^d	0.007	FMDL $f_{\text{mod.}} = 4 \text{ Hz}^e$
<i>Mus musculus</i>	Ehret 1975	18.5 ^a	60 dB ^c	157	0.008	DLF
<i>Cavia porcellus</i>	Heffner et al. 1971	18.5 ^a	30 dB ^c	647	0.035	DLF
<i>Tadarida brasiliensis</i>	Bartsch and Schmidt 1993	18.5 ^a	60 dB SPL	851 ^d	0.046	FMDL $f_{\text{mod.}} = 20 \text{ Hz}^e$
<i>Rhinolophus ferrumequinum</i>	Heilmann-Rudolf 1984	23 ^b	80 dB SPL	46	0.002	DLF
<i>Phyllostomus discolor</i>	present study	18.5	55 dB ^c	$\bar{x} = 95^d$ min. = 80 ^d	0.005 0.004	FMDL $f_{\text{mod.}} = 10 \text{ Hz}^e$
<i>Tursiops truncatus</i>	Herman and Arbeit 1972	19	43 dB (re 1 μbar)	84 ^d 45 ^d	0.004 0.002	FMDL $f_{\text{mod.}} = 1 \text{ Hz}$ $f_{\text{mod.}} = 2 \text{ Hz}^e$

^a Δf value for this frequency was calculated from Weber ratios (WR) or graphs given by the particular authors

^b due to the pronounced non-linearities in the cochlea of CF-FM bats an arithmetical transformation of measured thresholds to the lower frequency of 18.5 kHz was avoided

^c sensation level

^d = $2\Delta f$ (\rightarrow FMDL method)

^e modulation frequency at which lowest FMDLs were obtained for the particular species

Species comparisons

The difference limens obtained in the present study fit well with the general mammalian scheme. For interspecies comparisons, Weber ratios (WRs) are advantageous since, as mentioned above, thresholds obtained at different frequencies can be related to one another. According to Heffner et al. (1971), in mammals average WRs range between 0.004 (man) and 0.063 (white rat) with minimum values (DLFs) of 0.002 obtained in man. Correspondingly, in *P. discolor* (present study) a minimal WR of 0.002 was measured in bat 2 at a carrier frequency of 9 kHz and a modulation frequency of 100 Hz. A detailed comparison of difference limens from various mammalian species, including more recent studies on bats, is given in Table 1. In *Tursiops truncatus*, FMDLs were determined at nearly the same frequency as described here for *P. discolor*. Except for *Rhinolophus ferrumequinum* (see notes to Table 1), all other difference limens were standardized with respect to stimulus or carrier frequency. In the case of man, *Tadarida brasiliensis*, and *T. truncatus*, FMDLs are related to the ideal modulation frequency for the particular species. The resulting data (Table 1) reveal some remarkable trends. In the echolocating species *R. ferrumequinum*, *P. discolor*, and *T. truncatus*, the lowest WRs so far measured in any mammalian species have been found. Next to these animals, humans seem most sensitive in a frequency discrimination task. Within rodents, both a rather human-like sensitivity (*Mus musculus*) and also a quite poor performance have been found (*Cavia porcellus*). According to Bartsch and Schmidt (1993), in *T. brasiliensis* the ability to echolocate is not reflected in the species' FMDLs. Thus, the thresholds obtained from this bat are more comparable

to difference limens determined in guinea pigs than to those of other echolocating animals. In *T. truncatus* a shift in modulation frequency from 2 Hz to 1 Hz resulted in a substantial increase of FMDLs (Table 1). Since in *P. discolor* these low modulation frequencies have not yet been tested, lowering the modulation frequency further might have resulted in FMDLs equaling those of dolphins. The pronounced sensitivity of *R. ferrumequinum* in a frequency range far beyond the auditory fovea (Neuweiler 1970, 1990) of this CF-FM-bat seems unusual. However, in the frequency range of the CF-component of the species' echolocation calls, minimum WRs of 0.0005 were obtained (Heilmann-Rudolf 1984). According to Long (1994), frequency discrimination appears to be a difficult task to learn and thus the animals will use any other cues available to detect or discriminate sounds of importance. This means that, when tests are run in a frequency region where the audiogram slopes steeply (or the sound delivery system changes rapidly), the animals may use differences in loudness (Long 1994) instead of performing a frequency discrimination. Since corresponding steep slopes were actually found at frequencies adjacent to those represented in the center of the auditory foveal region of horseshoe bats (Neuweiler 1970), one can not exclude this possibility. Further, Bartsch and Schmidt (1993) stated that the exceptionally low FMDLs of *R. ferrumequinum* (Heilmann-Rudolf 1984) might have been influenced by the perception of roughness (for details see Zwicker and Fastl 1990) due to the temporal pattern of stimulus presentation (short pulses delivered at a high repetition rate).

The FMDLs determined in the present study are strongly dependent on the signal's modulation frequency. This is consistent with findings in other

mammals. In man, the hearing system is most sensitive to modulation frequencies around 4 Hz (Zwicker and Fastl 1990). From a modulation frequency of 10 Hz onwards, $2\Delta f$ values increase markedly with a slope corresponding to the square root of modulation frequency until a maximum is reached. Beyond this maximum, thresholds decrease again. The position of this maximum depends on the carrier frequency. The higher the signal's carrier frequency the higher is the modulation frequency leading to a maximum of $2\Delta f$. For example, FMDLs obtained at a carrier frequency of 8 kHz increase up to a modulation frequency of 300 Hz and then decrease (Zwicker and Fastl 1990). These authors relate the decrease in FMDLs at higher modulation frequencies to the perception of spectral sidebands which are characteristic of SFM stimuli (for details see Yost and Sheft 1993). These sidebands are spaced at integer multiples of the modulation frequency around the carrier frequency and may become audible separately at high frequencies of modulation. In the present study, bat 2 might have listened for additional sidebands at modulation frequencies beyond 500 Hz since the corresponding FMDLs tended to decline. In contrast, the high FMDL from bat 1 measured at a modulation frequency of 2000 Hz suggests that this animal was still performing on the base of frequency changes. Correspondingly, an increase in average FMDLs up to a modulation frequency of 2000 Hz was found in *T. brasiliensis* (Bartsch and Schmidt 1993).

Since the peripheral auditory system of FM-bats lacks the unique structural and physiological features which lead to an unequal representation of frequency in CF-FM-bats (Pollak and Casseday 1989), FM-bats should behave as other mammals and show a rather constant ratio of $2\Delta f/f$ throughout the hearing range. The determination of FMDLs at various carrier frequencies throughout a substantial part of the lesser spear-nosed bat's hearing range (present study) offers first behavioral evidence for a linear correlation between both parameters in this group of bats (Fig. 4). According to the course of the computed regression line (Fig. 4) which intersects the abscissa at a positive x-value, a non-linear relationship for low frequencies is expected. Correspondingly, in other mammals including man, $2\Delta f$ increases beyond a certain low frequency (about 500 Hz in man) in proportion to the carrier frequency but is nearly constant at lower frequencies (Ehret 1977; Zwicker and Fastl 1990).

Frequency modulation and species-specific calls

FMDLs determined in the range of modulation frequencies corresponding to those found in natural directive and isolation calls (Fig. 3) show no obvious peculiarities. However, since the average duration of maternal directive calls is less than 50 ms (Esser and Schmidt 1989) there should be a conflict between in-

creasing the frequency resolution ability by lowering the modulation frequency and the amount of transmittable information which seems at least partly encoded in the number of modulation cycles per call (Esser and Schmidt 1989). Considering that in *P. discolor* individuality and relatedness are encoded in *periodic* frequency modulations, the band of modulation frequencies used for intraspecific acoustic communication seems ideal since, due to the calls' short duration, periodic frequency modulations can not be realized below modulation frequencies of about 40 Hz. Further, for modulation frequencies higher than about 100 Hz, mean FMDLs tend to rise more steeply than for those below.

With regard to the behavioral performance of the bats, other correlations with natural call features become obvious. In bat 3, a FMDL could be obtained only at a modulation frequency of 100 Hz. Since in *P. discolor* the modulation frequencies of adult directive calls are restricted to the small band of 76–100 Hz there might be a correlation between performing the task and the similarity of artificial stimuli to natural calls. Further, in bat 1, FMDLs could be obtained for all tested modulation frequencies at carrier frequencies of 18.5 and 37 kHz which correspond to the average carrier frequencies of the fundamental and the first harmonic of maternal directive calls. In contrast, the same animal was unable to perform in a similar discrimination task at carrier frequencies of 9 and 74 kHz. In natural directive calls of *P. discolor* (Esser, unpublished data) the corresponding spectral components are absent (9 kHz; subharmonic) or at least reduced in intensity (74 kHz; 4th harmonic) in relation to more prominent call structures (harmonics 1–3).

Experiments related to echolocation behavior

In the context of bat echolocation the frequency discrimination ability of the auditory system seems of particular importance in those species using CF-FM-orientation calls. Insonified fluttering targets introduce periodic frequency *and* amplitude modulations to the CF-components of the returning echos. These acoustic glints (Schnitzler et al. 1983) enable the bats to detect flying insects even in the presence of massive echo clutter from the environment (e.g. Neuweiler 1990). In a variety of behavioral experiments CF-FM-bats were trained to detect an oscillating target or to discriminate between different ones (e.g. *R. ferrumequinum*, Schnitzler and Fliieger 1983; *Hipposideros speoris* and *H. lan-kadiva*, von der Emde and Schnitzler 1986). Using simulated insect echos von der Emde and Menne (1989) could show that *R. ferrumequinum* is able to discriminate between wing beat frequencies differing by only 2.8–4.6 Hz or by 5.6–9.2%. In a subsequent study (von der Emde and Schnitzler 1990) *R. ferrumequinum* discriminated successfully between phantom echos of

different insect species with identical wing beat frequencies. This performance persisted even when previously unknown echos from exactly the same insects (or from individuals of the same species) but from different angular orientations were presented. Further, removing the glints from the presented echos or the echo structures between glints revealed that the latter were used by the bats as a major source of information (von der Emde and Schnitzler 1990). Sum and Menne (1988) were able to show that FM-bats (*Pipistrellus stenopterus*) performed comparably to CF-FM-bats in an experiment where artificial fluttering targets should be discriminated. The authors compared the duty cycles in *P. stenopterus* ($\leq 3\%$) and in *R. ferrumequinum* (40–70%, von der Emde and Menne 1989) and stated that in *P. stenopterus* it is most likely that, due to the short duration of FM-echolocation calls, the cues for discrimination derive from echos of single calls. In contrast, the longer CF-FM-calls of *R. ferrumequinum* were even suited to cover successive insect wing beat cycles (von der Emde and Schnitzler 1990). However, recent single unit recordings from the inferior colliculus of *Myotis lucifugus* (Feng et al. 1994) suggest that even FM-bats might use the amplitude modulation pattern across a train of sound pulses for prey discrimination. However, this stroboscopic hearing model proposed by Feng et al. (1994) is based solely on amplitude cues. Also, from the phantom or fluttering target experiments cited above it can not be decided finally whether amplitude or frequency cues alone or a combination of both are more likely involved in the particular discrimination processes. Thus, the comparability of the corresponding detection thresholds with the FMDLs determined in the present study is very limited.

From another set of behavioral experiments related to echolocation in bats, rough estimates of WRs are available for *Eptesicus fuscus* and *Noctilio albiventris* (Roverud and Rabitoy 1994). In the case of *E. fuscus*, the ability to discriminate between artificial FM-echos and FM-like echos constructed from a series of brief, pure tone steps was determined (Roverud and Rabitoy 1994). In *N. albiventris* (Roverud 1994), a similar experimental paradigm was used but, according to the structure of the species' echolocation calls, a CF-component preceded the artificial FM- or FM-like echo part (for a critical view on the terminology of call components in this species see Neuweiler and Fenton 1988). The estimates of WRs for *E. fuscus* (0.009) and *N. albiventris* (0.0015) were calculated on the basis of the minimum Δf between resolvable, equidistantly spaced tone steps and the center frequency of the FM-like echo or echo part. However, since in this situation the threshold Δf should become first perceptible at the lower frequency limit of the FM-like signal (Zwicker and Fastl 1990; Moore 1993) these estimates of WRs seem questionable and too small and thus should be confirmed by more conventional psycho-

acoustic procedures (Bartsch and Schmidt 1993, present study) for both species.

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References

- Bartsch E, Schmidt S (1992) Frequency modulation thresholds in the free-tailed bat, *Tadarida brasiliensis*. In: Elsner N, Richter DW (eds) Rhythmogenesis in neurons and networks. Thieme, Stuttgart, pp 231
- Bartsch E, Schmidt S (1993) Psychophysical frequency modulation thresholds in a FM-bat, *Tadarida brasiliensis*. *Hearing Res* 67: 128–138
- Ehret G (1975) Frequency and intensity difference limens and nonlinearities in the ear of the housemouse (*Mus musculus*). *J Comp Physiol* 102: 321–336
- Ehret G (1977) Comparative psychoacoustics: perspectives of peripheral sound analysis in mammals. *Naturwissenschaften* 64: 461–470
- Emde G von der, Menne D (1989) Discrimination of insect wing-beat-frequencies by the bat *Rhinolophus ferrumequinum*. *J Comp Physiol A* 164: 663–671
- Emde G von der, Schnitzler H-U (1986) Fluttering target detection in Hipposiderid bats. *J Comp Physiol A* 159: 765–772
- Emde G von der, Schnitzler H-U (1990) Classification of insects by echolocating greater horseshoe bats. *J Comp Physiol A* 167: 423–430
- Emmons LH, Feer F (1990) Neotropical rainforest mammals: a field guide. The University of Chicago Press, Chicago
- Esser K-H (1994) Audio-vocal learning in a non-human mammal: the lesser spear-nosed bat *Phyllostomus discolor*. *NeuroReport* 5: 1718–1720
- Esser K-H, Daucher A (1996) Hearing in the FM-bat *Phyllostomus discolor*: a behavioral audiogram. *J Comp Physiol A* 178: 779–785
- Esser K-H, Schmidt U (1989) Mother-infant communication in the lesser spear-nosed bat *Phyllostomus discolor* (Chiroptera, Phyllostomidae) - evidence for acoustic learning. *Ethology* 82: 156–168
- Esser K-H, Schmidt U (1990) Behavioral auditory thresholds in neonate lesser spear-nosed bats, *Phyllostomus discolor*. *Naturwissenschaften* 77: 292–294
- Fay RR (1974) Auditory frequency discrimination in vertebrates. *J Acoust Soc Am* 56: 206–209
- Fay RR (1988) Hearing in vertebrates: a psychophysics databook. Hill-Fay Associates, Winnetka, Illinois
- Feng AS, Condon CJ, White KR (1994) Stroboscopic hearing as a mechanism for prey discrimination in frequency-modulated bats? *J Acoust Soc Am* 95: 2736–2744
- Fenton MB (1985) Communication in the Chiroptera. Indiana University Press, Bloomington
- Heffner R, Heffner H, Masterton B (1971) Behavioral measurements of absolute and frequency-difference thresholds in guinea pig. *J Acoust Soc Am* 49: 1888–1895
- Heilmann-Rudolf U (1984) Das Frequenzunterscheidungsvermögen bei der Großen Hufeisennase *Rhinolophus ferrumequinum*. Ph D thesis, Eberhard-Karls-University Tübingen, Tübingen (FRG)

- Herman LM, Arbeit WR (1972) Frequency difference limens in the bottlenose dolphin: 1–70 KC. *J Aud Res* 2: 109–120
- Keidel WD (1975) *Physiologie des Gehörs*. Thieme, Stuttgart
- Long GR (1994) Psychoacoustics. In: Fay RR, Popper AN (eds) *Comparative hearing: mammals*. Springer, New York, pp 18–56
- Moore BCJ (1993) Frequency analysis and pitch perception. In: Yost WA, Popper AN, Fay RR (eds) *Human psychophysics*. Springer, New York, pp 56–115
- Neuweiler G (1962) Bau und Leistung des Flughundauges (*Pteropus giganteus gig.* Brünn.). *Z Vergl Physiol* 46: 13–56
- Neuweiler G (1970) Neurophysiologische Untersuchungen zum Echoortungssystem der Großen Hufeisennase, *Rhinolophus ferrumequinum*. *Z Vergl Physiol* 67: 273–306
- Neuweiler G (1990) Auditory adaptations for prey capture in echolocating bats. *Physiol Rev* 70: 615–641
- Neuweiler G, Fenton MB (1988) Behaviour and foraging ecology of echolocating bats. In: Nachtigall PE, Moore PWB (eds) *Animal sonar: processes and performance*. Plenum Press, New York, pp 535–549
- Penner MJ (1995) Psychophysical methods. In: Klump GM, Dooling RJ, Fay RR, Stebbins WC (eds) *Methods in comparative psychoacoustics*. Birkhäuser, Basel, pp 47–57
- Pollak GD, Casseday JH (1989) *The neural basis of echolocation in bats*. Springer, Berlin Heidelberg New York
- Roverud RC (1994) Complex sound analysis in the lesser bulldog bat: evidence for a mechanism for processing frequency elements of frequency modulated signals over restricted time intervals. *J Comp Physiol A* 174: 559–565
- Roverud RC, Rabinoy ER (1994) Complex sound analysis in the FM bat *Eptesicus fuscus*, correlated with structural parameters of frequency modulated signals. *J Comp Physiol A* 174: 567–573
- Schnitzler H-U, Flieger E (1983) Detection of oscillating target movements by echolocation in the greater horseshoe bat. *J Comp Physiol* 153: 385–391
- Schnitzler H-U, Menne D, Kober R, Heblich K (1983) The acoustical image of fluttering insects in echolocating bats. In: Huber F, Markl H (eds) *Neuroethology and behavioral physiology*. Springer, Berlin Heidelberg New York, pp 235–250
- Sum YW, Menne D (1988) Discrimination of fluttering targets by the FM-bat *Pipistrellus stenopterus*. *J Comp Physiol A* 163: 349–354
- Yost WA, Sheft S (1993) Auditory perception. In: Yost WA, Popper AN, Fay RR (eds) *Human psychophysics*. Springer, New York Berlin Heidelberg, pp 193–236
- Zwicker E, Fastl H (1990) *Psychoacoustics: facts and models*. Springer, Berlin Heidelberg New York