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Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication

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Abstract We investigated the relationship between auditory sensitivity, frequency selectivity, and the vocal repertoire of greater spear-nosed bats (*Phyllostomus hastatus*). *P. hastatus* commonly emit three types of vocalizations: group-specific foraging calls that range from 6 to 11 kHz, low amplitude echolocation calls that sweep from 80 to 40 kHz, and infant isolation calls from 15 to 100 kHz. To determine if hearing in *P. hastatus* is differentially sensitive or selective to frequencies in these calls, we determined absolute thresholds and masked thresholds using an operant conditioning procedure. Both absolute and masked thresholds were lowest at 15 kHz, which corresponds with the peak energy of isolation calls. Auditory and masked thresholds were higher at sound frequencies used for group-specific foraging calls and echolocation calls. Isolation calls meet the requirements of individual signatures and facilitate parent-offspring recognition. Many bat species produce isolation calls with peak energy between 10 and 25 kHz, which corresponds with the frequency region of highest sensitivity in those species for which audiogram data are available. These findings suggest that selection for accurate offspring recognition exerts a strong influence on the sensory system of *P. hastatus* and likely on other species of group-living bats.

Keywords Chiroptera · Frequency discrimination · Hearing · Recognition signals · Social communication

Introduction

For communication systems to function effectively, recognition signals or signatures must contain information about identity, and receivers must be able to detect, as well as discriminate among those signatures. Perception of individual signatures is believed to occur through a template-matching process, a mechanism by which a template of the target signal is formed in the memory of the receiver, and new signals are then compared with this template (Holmes and Sherman 1982; Lacy and Sherman 1983). The difficulty of this task depends on the number of entities in the recognition pool and the nature of the decision. As the number of entities increases, the amount of information that must be encoded by the signaler and decoded by the receiver must increase to insure accurate recognition (Beecher 1989). Thus, the ability to recognize a signaler depends on the task, the resolving power of the receiver, and the similarity between the template and novel signal.

Most empirical studies of signature systems have focused on a single perceptual task, such as offspring recognition by a parent, and have frequently demonstrated that sufficient information exists in the signal to permit accurate identification (birds, McArthur 1982; Stoddard and Beecher 1983; Nakagawa et al. 2001; seals, Trillmich 1981; Insley 2001; primates, Pereira 1986; dolphins, Smolker et al. 1993). An issue that has received considerably less attention is how a sensory system should be designed when more than one type of recognition problem must be solved. An ideal system would have sufficient sensitivity and resolving ability to enable accurate detection and discrimination of all possible signal variants. However, animals are constrained by the physics associated with signal production and transmission, as well as by physiological limitations imposed on the receiver (Bradbury and Vehrencamp 1998).

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Greater spear-nosed bats (*Phyllostomus hastatus*) present an important case for the study of signal production and reception because they use vocalizations for three different recognition problems: to recognize social group membership, to recognize offspring, and to recognize self-generated sonar vocalizations from echoes and calls produced by conspecifics.

P. hastatus roosts in stable social groups of, on average, 20 unrelated females (McCracken and Bradbury 1981) that appear to use group-specific "screech" calls to coordinate foraging (Boughman 1997; Boughman and Wilkinson 1998; Wilkinson and Boughman 1998). Auditory specializations might occur in the frequency range of screech calls because these low-frequency (5–12 kHz) signals can be modified by vocal learning (Boughman 1998). When separated from their mothers, infant *P. hastatus* emit isolation calls that attract adult females and facilitate maternal retrieval of offspring. Isolation calls consist of a harmonic series of frequency modulated tones that range from 15 to 100 kHz (Gould 1975). Isolation calls contain sufficient variation in frequency and temporal characteristics to permit unambiguous assignment of calls to individuals (D.L. Lill and G.S. Wilkinson, unpublished data). As in other species that roost in large colonies, recognizing and directing parental care towards young should be under strong selection (Beecher et al. 1981; Beecher 1982; Colgan 1983). Hearing in the frequency range of isolation calls should, therefore, also be under selection to the extent that it aids in detecting the calls of fallen offspring and discriminating among related and unrelated individuals.

P. hastatus emit short (1–3 ms), low amplitude echolocation calls which consist of high frequency (80–40 kHz), broad band sweeps (Griffin and Novick 1955; Pye 1967). *P. hastatus* are omnivorous, predominantly consuming fruit and large insects (Emmons 1997). It has been long recognized that *P. hastatus* use echolocation for orientation as do most frugivorous phyllostomids; however, recent studies have shown that *P. hastatus* also rely on echolocation to find fruit (Kalko and Condon 1998). Thus, perception of sonar cries and returning echoes should also be under selection.

In bats, studies on hearing have focused mainly on the ultrasonic frequency range, even though many species are most sensitive to frequencies below those used for echolocation (reviewed in Neuweiler 1990; Moss and Schnitzler 1995). Low-frequency hearing may be used for passive listening to prey-generated noises (Ryan et al. 1983; Coles et al. 1989; Schmidt et al. 1991; reviewed in Neuweiler 1990), however, a correspondence between frequencies of highest auditory sensitivity and social vocalizations has been noted for some species (*Noctilio leporinus*, Wenstrup 1984; *Macroderma gigas* and *Nyctophilus gouldi*, Guppy and Coles 1988; *Phyllostomus discolor*, Esser and Daucher 1996). Except for work on *P. discolor* (Esser and Kiefer 1996), few studies have focused on possible auditory specializations related to conspecific vocal signals in bats.

In this study we examine auditory sensitivity and frequency selectivity in *P. hastatus* and compare these estimates with the spectral content of both social communication and echolocation signals. We use an operant conditioning paradigm to determine hearing sensitivity and frequency selectivity. We estimate frequency selectivity by measuring critical ratios from measurements of pure-tone thresholds embedded in broadband white noise. Critical ratios indirectly measure the frequency selectivity of the auditory system, which operates with a bank of overlapping band-pass filters or critical bands (Fletcher 1940). Estimates of critical bands from critical ratios are based on the following assumptions: (1) the detection of pure tones embedded in broadband noise are masked only by the noise within the critical band; (2) critical bands are symmetrical and rectangular; and (3) the energy level of the tone at threshold is equal to the energy level of the noise within the critical band (Fletcher 1940). From these assumptions it follows that the critical ratio in decibels can be used to estimate the critical band at a given frequency by determining the bandwidth of the white noise that contains energy equal to that of the level of the tone at threshold. Smaller critical ratios imply narrower frequency bands and higher frequency selectivity.

In our study we use the same experimental set up and positive reinforcement methods to generate an audiogram and a critical ratio function for four individual *P. hastatus*. Absolute hearing sensitivity measured in this study can be compared with published audiograms obtained using negative reinforcement (Koay et al. 2002) and neural recordings (Grinnell 1970). Our data permit direct comparisons of hearing sensitivity and frequency selectivity because we use the same behavioral methods in the same individual bats. We then compare these measures to the spectral content of three common vocalizations: screech calls, isolation calls, and echolocation calls.

Materials and methods

Subjects

Four adult female *P. hastatus* were used in the experiments. The experimental animals came from groups captured in Trinidad, West Indies in 1993. During the study, bats were housed in a large cage (3.3 m×2.7 m×2.4 m) in a separate room at the University of Maryland, College Park. The room was maintained on an 8-h light, 16-h dark cycle at approximately 24°C and 30% humidity. Bats were maintained at a weight of 65–70 g during experiments (85–90% free-fed body weight) on a diet of fruit and marmoset food (Premium Nutritional Products) supplemented with mealworms, which were provided as food rewards during test sessions.

Behavioral training

All experiments were conducted in a single-wall acoustic chamber (Industrial Acoustics Company) lined with acoustic foam (Sonex). Bats were trained and tested using a V-shaped platform enclosed in a hardware-cloth cage (Fig. 1). Subjects were trained for a modified

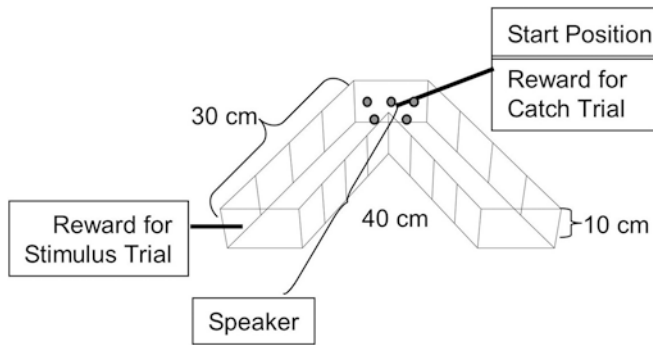


Fig. 1 Schematic of test apparatus (not to scale). During stimulus trials bats crawled to the end of the platform and were presented with a mealworm. During catch trials bats were presented with a mealworm for remaining at the start position. Grey circles indicate the five positions where stimulus levels were recorded

go/no-go procedure (Suthers and Summers 1980). A red light was used to signal the onset of a trial. During each trial either a pure tone was played (stimulus trial) or was not played (catch trial). The bats were rewarded with a mealworm at the end of the right arm of the platform (30 cm long by 13 cm wide) during stimulus trials and a mealworm at the starting position during catch trials. If the bats went to the end of the platform during catch trials, the light was extinguished and both verbal commands (“get back”) as well as light tapping on the bottom of the cage were used to direct bats back into the starting position. If bats did not move to the end of the platform during stimulus trials, a 20-s time-out was given. If the bat failed to respond for three consecutive trials, the session was terminated.

Once the subjects learned the go/no-go task, a one-up/one-down staircase procedure was introduced to the stimulus levels (Niemic and Moody 1995). The percentage of catch trials was varied in order to maximize correct responses to stimuli while keeping incorrect responses to catch trials at or below 20%. In order to be certain that the bats were accustomed to the procedure and performing reliably, we did not begin collecting audiogram measurements until the thresholds for all four bats at 10 kHz were within 5 dB of each other over five consecutive sessions.

Threshold determination

During test sessions, stimulus (65%) and catch (35%) trials were alternated at random. Sessions with greater than 25% response during catch trials were discarded, although false alarm rates were usually below 10%. If a bat responded correctly to a stimulus, the amplitude of the signal was reduced by 5 dB. If a bat failed to respond to a stimulus for two consecutive trials, the amplitude of the tone was increased by 5 dB. For each session, trials continued until six reversals occurred. A reversal was counted every time the direction of amplitude adjustment was changed. The first two reversals were discarded and the last four averaged to calculate a threshold as described in Niemic and Moody (1995).

For the audiogram, thresholds were determined for each subject at 11 different frequencies (2.5, 5, 7.5, 10, 15, 20, 30, 40, 60, 80 and 100 kHz). For critical ratio estimates, masked thresholds were determined for 7.5, 15, 30, 40 and 60 kHz with a noise spectrum level of 25 dB Hz⁻¹. We also measured thresholds at 7.5, 15 and 40 kHz with a noise spectrum level of 35 dB Hz⁻¹. Valid critical ratios should remain the same at different noise spectrum levels (Fletcher 1940). Threshold and masked threshold estimates were taken at least three times at each test frequency. Critical ratios were calculated as the amplitude (in dB) of the tone at threshold minus the spectrum level of the noise (in dB Hz⁻¹). Based on assumptions outlined by Fletcher (1940), each critical ratio was converted to an equivalent filter bandwidth using the formula: critical ratio (Hz) = 10^(critical ratio dB/10).

Stimuli and calibration

All pure tone signals were synthesized digitally at a sample rate of 250 kHz using SIGNAL (Version 3.0, Engineering Design). Every stimulus trial consisted of three pure tones with durations of 350 ms each, including 25-ms rise/fall times and 50-ms intervals between tones. Stimuli were played through two serially connected attenuators (Hewlett Packard 350D) that controlled amplitude in 5-dB steps. The signal was then band pass filtered (Krohn-Hite 3550), amplified (Harman Kardon AVR 100), and sent to a speaker (Pioneer PT-R) that was located 1 m from the subjects' starting position. White noise was created using a function generator (Stanford Research Systems, DS345), passed through a graphic equalizer (Rack Rider, RR-131) and filtered (Stanford Research Systems, SR650). With this system we created random white noise that was flat (± 3 dB) from three to 80 kHz. For the masked thresholds experiments, pure tones and noise were sent to a custom made mixer prior to being amplified and sent to the speaker.

Each day we recorded the pure tones and/or noise at five locations separated by 2 cm at the bats' starting position on the platform (Fig. 1). Sounds were recorded onto a laptop computer equipped with a high-speed data acquisition card (INEES, Daq508), which sampled 16 bits at 333 kHz, using a one-eighth inch microphone (Brüel and Kjaer), connected to a preamplifier (Larson Davis 2200C) and amplifier (SHURE, FP-2). Time waveforms and power spectra of stimuli were inspected daily for any distortions using Bat Sound Pro (Pettersson Elektronik). We also recorded a calibration tone daily with a piston phone (Brüel and Kjaer type 4231). Sound levels were calculated by taking the root mean square of 10,000 samples of each waveform and then averaged over the five locations on the observation platform.

Vocalizations

All *P. hastatus* vocalizations, except for echolocation calls (see below), were recorded at Guanapo cave, Trinidad (McCracken and Bradbury 1981), in April 2001. We recorded screech calls from flying bats at the entrance of the cave using a shotgun microphone (Audio-Technica AT4071A) and phantom power supply (AKG Acoustics B18) connected to a laptop computer which sampled 16 bits at 44 kHz. This system had a flat response (± 5 dB) from 20 Hz to 20 kHz. Screech calls do not contain appreciable energy above 15 kHz (Boughman 1997).

We recorded isolation calls from ten individual pups that were captured with their mothers and briefly held outside Guanapo cave in April 2002. Isolation calls were recorded at a sample rate of 250 kHz using a high-frequency microphone (Ultra Sound Advice M2) and the same equipment that was used for making recordings during psychoacoustic experiments.

The four bats studied in the psychoacoustic experiments were allowed to fly freely in a large room at the University of Maryland. Echolocation calls were recorded with a high frequency microphone (Ultra Sound Advice M2), band pass filtered (5–110 kHz, Stewart, VBF7), amplified, and digitized onto a laptop computer using a high-speed analog-digital card which sampled 16 bits at 250 kHz (IOTECH Wavebook).

We calculated mean power spectra for 23 screech calls, 50 isolation calls (five calls/pup) and 50 echolocation calls using Bat Sound Pro (Pettersson Electonik). For each call type we determined the peak frequency and calculated the peak frequency range by determining frequencies above and below the peak frequency that were -3 dB below the peak energy. For each call type we determined the relative amplitude of the power spectrum at each frequency for which we had measured an auditory threshold, except for screech calls where only frequencies equal to or below 20 kHz were included in the analysis. as this was the upper range of the microphone used to record these calls. For each call type we calculated a correlation coefficient between the spectral power of the vocalization and the mean of the lowest auditory thresholds measured in each bat. Because the data were not normally distributed and violated independence assumptions, we tested whether the

correlation coefficients were different from zero using randomization tests (Manly 1991). For each call type, the order of one variable was randomized and a correlation coefficient was calculated. Correlation coefficients were calculated for all possible permutations for screech calls ($n=720$) and for 10,000 permutations for both echolocation and isolation calls. We then determined the proportion of these correlation coefficients that had absolute values greater than the observed correlation to assign a two-tailed probability to the hypothesis that the observed coefficient was significantly different from zero.

Results

Absolute thresholds

All four bats responded to tones from 2.5 to 100 kHz. Pure-tone thresholds were similar for all four bats and were lowest at 15 kHz (Fig. 2a). Thresholds ranged from a maximum of 71 dB SPL at 2.5 kHz to a minimum of 13 dB SPL at 15 kHz. Hearing sensitivity increased at a rate of approximately 5 dB kHz^{-1} from 5 to 15 kHz and then decreased at a slower rate of approximately 0.4 dB kHz^{-1} from 15 to 100 kHz.

Masked thresholds

Critical ratios were similar for all four bats and for the two noise spectrum levels tested (Figs. 2b, 3b). Critical ratio estimates were lowest at 15 kHz, increased by approximately 10 dB at 30 kHz, decreased slightly by

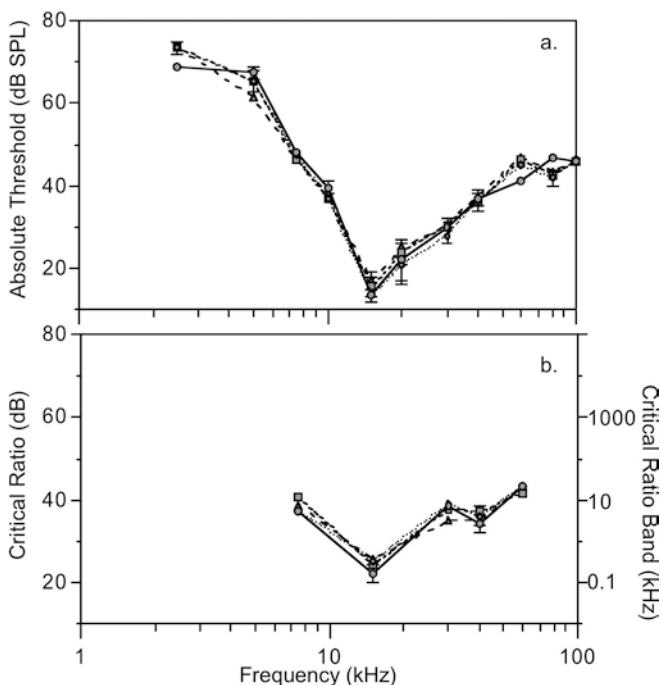


Fig. 2 Average of the lowest two thresholds for each of the four bats: **a** absolute thresholds, **b** critical ratios at 25 dB Hz^{-1} . For critical ratios, estimates in decibels are on the left axis and equivalent critical ratio bands in Kilohertz are shown on the right axis

4 dB at 40 kHz, and then increased by another 10 dB at 60 kHz, where the highest estimates were obtained. Critical ratio values can be converted to equivalent frequency bands following Fletcher's (1940) assumptions. Calculations yield a minimum bandwidth of 209 Hz and maximum bandwidth of approximately 17 kHz (Fig. 3b).

Spectral characteristics of species-specific vocalizations

Screech calls are broadband acoustic signals (Fig. 4a) with average peak energy at 9.5 kHz and a -3 dB frequency range of 6–11 kHz (Fig. 3a). Isolation calls are characterized by frequency-modulated syllables with multiple harmonics (Fig. 4b). Maximum energy of these calls occurred in the fundamental at 15 kHz and the -3 dB frequency range was 14–17 kHz (Fig. 3a). The peak frequency of the first harmonic of isolation calls was at 28 kHz with a -3 dB frequency range of 26–29 kHz, which was 5 dB less than the peak frequency at 15 kHz. Echolocation calls consist of steep multi-harmonic sweeps (Fig. 4c) with peak energy at 46 kHz and a -3 dB frequency range between 42 and 50 kHz (Fig. 3a). A second peak occurs at 62 kHz and had a -3 dB range of 59–65 kHz.

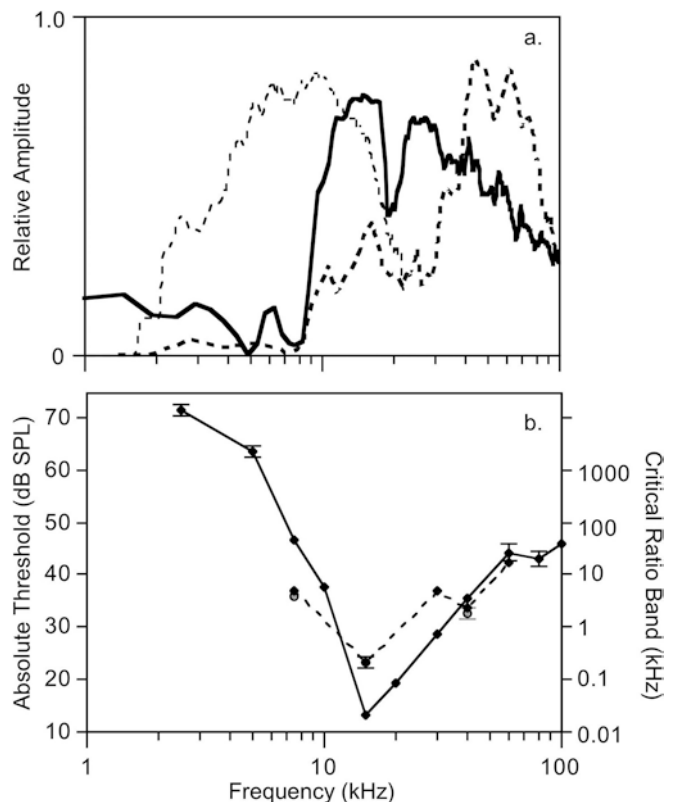


Fig. 3 a Average power spectra of screech (light dashed line), isolation (solid line) and echolocating calls (thick dashed line). **b** Average of the lowest absolute thresholds (solid line) and masked thresholds at 25 dB Hz^{-1} (dashed line) and 35 dB Hz^{-1} (grey circles) for all four bats

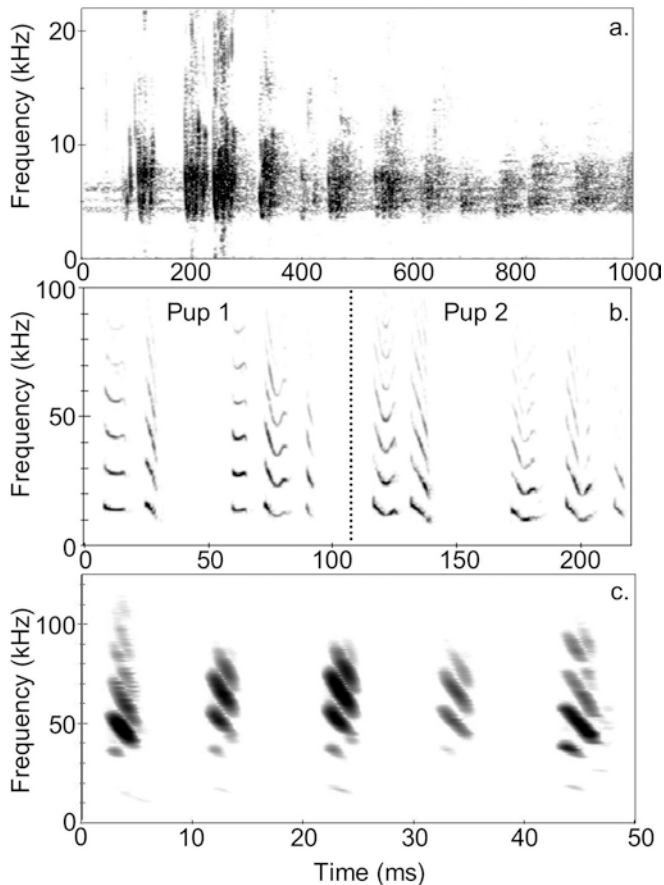


Fig. 4 **a** Spectrogram of screech calls recorded in flight outside of Guanapo cave. **b** Spectrogram of isolation calls made by two pups at Guanapo cave. Two of the four call types (double-note and triple-note) are shown for each pup. **c** Spectrogram of echolocation calls recorded in a flight room

The peak energy of screech calls (6–11 kHz) lies in a frequency region where hearing sensitivity is comparatively poor (Fig. 3). The correlation between power spectra of screech calls and absolute auditory thresholds was not significant (Fig. 3, $r=0.057$, randomization test $P=0.911$, $n=6$). In contrast, peak energy of isolation calls (14–17 kHz) is within the range of best hearing sensitivity, and we found a significant negative correlation between isolation call power spectra and auditory thresholds across sound frequency (Fig. 3, $r=-0.790$, randomization test $P=0.004$, $n=11$). There was no relationship between the spectral characteristics of echolocation calls and absolute hearing sensitivity ($r=-0.318$, randomization test $P=0.356$, $n=11$).

Critical ratios followed pattern similar to that of absolute thresholds with highest frequency selectivity at 15 kHz, corresponding with the peak frequency of isolation calls. Critical ratio measurements resulted in estimated bandwidths of approximately 5 kHz in the frequency range of screech calls, 200 Hz at the peak frequency of isolation calls, and between 2 and 17 kHz in the range of echolocation calls. A small decrease in critical ratios occurred at 40 kHz, which was close to the peak frequency of echolocation calls. However, frequency

selectivity at peak frequencies of screech and echolocation calls is considerably less than it is at the frequencies containing maximum energy in isolation calls.

Discussion

Hearing sensitivity and frequency selectivity

In this study we examined hearing sensitivity and frequency selectivity of *P. hastatus* using positive reinforcement and a go/no-go procedure. In our behavioral audiogram, the minimum absolute threshold was 13 dB SPL at 15 kHz. Minimum absolute thresholds in bats have been reported at 0 dB SPL or lower using conditioned avoidance (e.g. *Eptesicus fuscus*, Koay et al. 1997), two alternative forced choice (*Megaderma lyra*, Schmidt et al. 1983), and neural recording methods (e.g. *M. lyra*, Kössl 1992; *M. gigas*, Guppy and Coles 1988). The higher thresholds we obtained were likely due to our training procedures, as we did not use negative reinforcement. Our audiogram showed greater sensitivity to low frequencies than the neurophysiological audiogram by Grinnell (1970). However, higher thresholds at frequencies below 25 kHz have been noted for most bat audiograms that were measured by neural recordings while animals were under anesthesia (Neuweiler 1990). The shape of our audiogram for *P. hastatus* is similar to a behavioral audiogram recently published by Koay et al. (2002), although they report a minimum threshold of 1.5 dB SPL at 20 kHz and a drop in threshold at 64 kHz, a frequency we did not test. Both absolute sensitivity and critical ratio data collected in this study showed consistent thresholds across days and bats, suggesting that our findings are reliable. Furthermore, because we used positive reinforcement, our thresholds should be representative of amplitude levels that would elicit behavioral responses under natural circumstances.

In most mammals and birds critical ratios increase by approximately 3 dB/octave over the range of hearing (Fay 1988; Dooling et al. 2000). *P. hastatus* deviates from this pattern with a 14 dB/octave decrease in critical ratios from 7.5 kHz to 15 kHz, followed by an approximate 10 dB/octave increase in critical ratios from 15 kHz to 60 kHz. Deviations from the general pattern have been reported in some birds with smaller critical ratios in the frequency range of vocalizations and have been interpreted as possible specializations for conspecific communication (Okanoya and Dooling 1987; Dooling et al. 2000; Wright et al. 2003). Interestingly, although critical ratios have only been published for two species of bats, both species deviate from the 3 dB/octave pattern (*Rhinolophus ferrumequinum*, Long 1977; *Rousettus aegyptiacus*, Suthers and Summers 1980).

Critical ratios can be used to estimate auditory filter bandwidths. The smaller the estimated auditory filter bands, the greater the animal's ability to discriminate between sound frequencies. Frequency discrimination, or the ability to detect changes in pure-tone frequency,

shows a parallel relationship with critical ratios in species for which both have been measured; however, critical ratios are usually 20 times larger than pure tone frequency discrimination thresholds (Long 1994). If this relationship holds in *P. hastatus*, then frequency discrimination would be approximately 10 Hz, 250 Hz, and between 100 and 850 Hz in the range of isolation calls, screech calls and echolocation calls, respectively.

Screech calls

The spectral energy of screech calls between 6 and 11 kHz lies near the lower frequency limit of hearing in *P. hastatus*. Although the peak energy in these calls does not correspond with peak hearing sensitivity, some energy at higher frequencies of screech calls overlaps with lower thresholds in the audiogram. However, screech calls are emitted while bats forage, which requires lower frequencies for optimal propagation over long distances (Wiley and Richards 1982). Estimates of screech call amplitude are 75–79 dB SPL at 1 m (J.W. Boughman, unpublished data), and when combined with hearing sensitivity and transmission loss (Marten et al. 1977), result in detection distances between 70 and 109 m (for 5 and 10 kHz, respectively).

Screech call discrimination should be an easier perceptual task than isolation call discrimination because the recognition problem requires less information. This is due to the fact that the number of groups likely encountered while foraging is fewer than the number of pups in a cave. Thus, heightened frequency selectivity may not be required to learn and decode these signals. Analysis of 161 calls from 28 bats in three groups revealed that seven acoustic features, including spectral, temporal, and relative amplitude variables exhibited significant variation among groups (Boughman 1997). Four of these variables involved frequency measurements and 14–34% of the variation in these variables was accounted for by group identity. In contrast, group identity only explained between 0 and 13% of the variation in each of four temporal variables. The most informative variable was bandwidth, which ranged from 5 to 8 kHz—surprisingly close to our critical ratio estimate at this frequency range. The frequency at –12 dB below the second energy peak of these calls was the second most informative variable. This variable lies between 10 and 12 kHz. Although we did not measure critical ratios at these frequencies, given the form of our critical ratio function, better frequency discrimination would be expected at these frequencies when compared with the first peak of the calls that lies between 4 and 5 kHz (Boughman 1997).

Echolocation calls

Greatest auditory sensitivity and frequency selectivity of *P. hastatus* did not correspond with the spectral peaks of echolocation calls at 42 and 60 kHz. Although there was

a small decrease in critical ratios at 40 kHz, frequency selectivity was much poorer than at the peak frequency of isolation calls. This result does not preclude other auditory specializations related to sonar localization. *P. hastatus* echolocation calls are broadband sweeps, well suited for carrying spatial information about target range, direction in azimuth and elevation (Simmons 1973; Simmons and Stein 1980). Heightened sensitivity and frequency selectivity in the ultrasonic range may not be essential for these tasks. For example, echolocating bats use a temporal cue, the time delay between sonar cries and returning echoes, to determine the distance to targets. Species using broadband signals, such as *P. hastatus*, exhibit finer range resolution than species using narrowband signals (Simmons 1973). In contrast, *R. ferrumequinum*, a bat that uses long constant frequency echolocation signals, has very low critical ratios in the frequency range of their sonar cries, indicating specialized frequency selectivity in that region (Long 1977). Long narrowband signals, such as those used by *R. ferrumequinum*, are well designed for spectral analysis but are poorly suited for temporal analysis (Simmons and Stein 1980).

Isolation calls

Both sensitivity and frequency selectivity were highest at 15 kHz, which corresponds with the peak energy of the fundamental in isolation calls. Auditory sensitivity was also high across the peak energy range of the first harmonic (26–30 kHz). Although additional harmonics exist in these calls, these higher harmonics contain less energy and attenuate more rapidly, making them less reliable for isolation call detection and discrimination (Wilkinson 1995). Although we do not have absolute amplitude measurements of these calls, they are emitted at least 12–14 dB louder than echolocation calls (Gould 1977). Highest auditory sensitivity at the peak frequency of isolation calls should maximize adult detection of offspring.

An association between auditory sensitivity, frequency selectivity and isolation calls, as well as maternal directive calls, has been reported in the congener, *P. discolor* (Esser and Daucher 1996; Esser and Lud 1997). In this species, young bats appear to modify isolation calls to match maternal directive calls (Esser 1994). Maternal directive calls have unique sinusoidal frequency modulation patterns (Esser and Schmidt 1989; Esser and Lud 1997). Studies in *P. discolor* on the minimum detectable frequency modulation (Esser and Kiefer 1996) and minimum detectable difference in modulation frequency (Esser and Lud 1997) were conducted at 18.5 kHz, the fundamental frequency of maternal directive calls. Results not only indicated sufficient spectral and spectro-temporal resolution to distinguish individuals but also enhanced frequency resolution when compared with other mammals (Esser and Kiefer 1996; Esser and Lud 1997).

Isolation call discrimination is likely among the most challenging acoustic tasks encountered by adult *P. hastatus* because they must discriminate among many isolation call signatures. The amount of variation among pups in acoustic features of isolation calls should reflect the magnitude of this problem (Wilkinson 2003). Nested analysis of variance on acoustic measurements of the first harmonic in 615 isolation calls recorded from 127 pups captured in 22 female groups, revealed that five frequency and five temporal variables exhibited significant variation among pups (D.L. Lill and G.S. Wilkinson, unpublished data). After adjusting for age-related effects, variation among pups accounted for 38–51% of the total variance in each of the five frequency measures and 27–39% of the variance in each of the five temporal measures. Heightened frequency selectivity should enable females to identify pups using such large acoustic differences.

Highest hearing sensitivity and frequency selectivity occurred at the same frequency and corresponded with peak spectral energy of isolation calls. This finding is consistent with auditory specializations for detection and discrimination of individual vocal signatures and indicates that isolation calls are an essential component of the vocal repertoire. Non-volant pups frequently fall from roosts and then emit isolation calls (J.W. Wilkinson and K.M. Bohn, unpublished data). Isolation calls attract females who carry young back to the roost. *P. hastatus* have low reproductive rates (one pup per year) and high infant mortality (40–60%, McCracken and Bradbury 1981; Stern and Kunz 1998). Pup recognition, therefore, is essential for successful reproduction. Detection and discrimination of isolation calls is likely important for many bat species that roost in colonies and leave non-volant young behind while foraging. Isolation calls exhibit the requirements of individual signatures in many species of bats (e.g. *Tadarida brasiliensis*, Gelfand and McCracken 1986; *P. discolor*, Rother and Schmidt 1985; *Nycticeius humeralis*, Scherrer and Wilkinson 1993; *Plecotus auritus*, de Fanis and Jones 1995) and maternal recognition of individual isolation calls has been demonstrated in a few species (Rother and Schmidt 1985; Balcombe 1990; de Fanis and Jones 1995). Furthermore, overlap between regions of peak sensitivity and isolation call frequencies occurs in several other bat species (e.g. *Antrozous pallidus* Brown and Grinnell 1980; *P. auritus*, Coles et al. 1989; de Fanis and Jones 1995; *R. ferrumequinum*, Long and Schnitzler 1975; Matsumura 1979), as well as marsupials (*Dasyurus hallucatus*, Aitkin et al. 1994; *Monodelphis domestica*, Frost and Masterton 1994; Aitkin et al. 1997) and rodents (*Mus musculus*, Ehret 1989). Thus, detection and discrimination of offspring vocalizations may represent an important source of selection on hearing sensitivity in a variety of mammals. These findings indicate that perception of social vocalizations, particularly those involved in parent-offspring communication, deserves further study.

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