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Passive sound-localization ability of the big brown bat (*Eptesicus fuscus*)

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Abstract

The passive sound-localization ability (i.e. minimum audible angle) of the big brown bat, *Eptesicus fuscus*, was determined using a conditioned avoidance procedure in which the animals were trained to discriminate left sounds from right sounds. The mean threshold of three bats for a 100-ms broadband noise burst was 14°, a value that is about average for mammals. A similar threshold of 15° was obtained for one animal when it was retested with one of its own recorded echolocation calls as the stimulus. The two bats tested on pure-tone localization were able to localize high-frequency, but not low-frequency tones, even when a low-frequency tone was amplitude modulated, a result indicating that these bats are not able to use binaural time-difference cues for localization. Finally, given the width of the bat's field of best vision, as determined by a count of its ganglion-cell density, its sound-localization acuity is consistent with the hypothesis that the role of passive sound localization is to direct the eyes to the source of a sound.

Key words: Bat; Binaural cue; *Eptesicus fuscus*; Evolution; Retinal ganglion cell; Sound localization

1. Introduction

All microchiropteran bats use active echolocation, and they do so by emitting a series of brief frequency-modulated, mostly ultrasonic, sounds and detecting the fine changes in the returning echoes that result from the reflection of the sound off a target. Some species from both the Old World (*Rhinolophus spp.*) and New World (*Pteronotus spp.*) have supplemented this frequency-modulated pulse with the addition of a long constant-frequency component (for a review of echolocation, see Neuweiler, 1989). In the case of big brown bats (*Eptesicus fuscus*) their frequency-modulated echolocation calls enable them to distinguish between objects based on size, shape, distance, direction, and motion, all accomplished by sensing the modifications of the emitted pulses that are reflected back to their ears (Simmons et al., 1975). They are able to do this because their auditory system, which although similar in its basic plan to that of other mammals, has evolved the ability to make fine discriminations of their returning echoes that are believed to be beyond the capacities of non-echolocators (Grinnell, 1995).

Given the specialization of bats for echolocation, the question arises as to how this ability might have affected the non-echolocating aspects of their hearing. In particular, because bats use active echolocation to locate objects in their environment, one might ask whether this has affected their ability to localize sounds produced by other animals. In other words, is their passive sound localization similarly acute? On the one hand big brown bats, using sonar, can distinguish targets that differ in their spatial separation by only 1.5°, suggesting that their nervous system can sustain very good sound localization (Simmons et al., 1983). On the other hand, it might be argued that because of their echolocation ability, they have little need for passive sound localization and should thus have poor localization acuity.

Currently, our knowledge of the passive sound-localization ability of big brown bats is based on field studies. These studies indicate that big brown bats tend to fly towards areas where natural environmental sounds

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are loudest and, presumably, concentrations of prey are the greatest (Buchler and Childs, 1981). However, such studies cannot tell us how accurate the bats are at localizing sound nor what sound-localization cues they use.

A knowledge of the passive sound-localization ability of echolocating bats is of interest for two reasons. First, such information has relevance to the anatomical and physiological study of sound localization in bats. Thus, a knowledge of the big brown bat's localization acuity and its use of binaural locus cues can help us understand the significance of anatomical variation in the brainstems of bats as well as the physiological response properties of auditory neurons (e.g. Grothe et al., 1996; Kuwabara and Zook, 1992). Second, the passive sound-localization acuity of bats is of interest to the comparative study of hearing in mammals. Specifically, it has been noted that the ability of mammals to localize sound is related to the width of their field of best vision. This relation is based on the role of sound localization in directing the gaze to the source of a sound (Heffner and Heffner, 1992b; Heffner et al., 1994). Although big brown bats roost and fly in lighted environments, retain functional vision, and have been observed to fly toward the glow of sunset (Buchler and Childs, 1982), they nevertheless rely primarily on echolocation rather than vision to navigate and capture prey. Thus the question arises as to whether they conform to the relationship between vision and sound localization established in non-echolocating mammals.

The present study was a four-fold investigation of the passive sound-localization ability of big brown bats. First, the animals' left-right sound-localization acuity was determined using a standard 100-ms noise burst as well as one of their echolocation calls. Second, their use of binaural intensity- and time-difference cues for localization was examined by determining their ability to localize pure tones at a fixed angle of 60° horizontal separation. Third, an estimate of the interaural intensity difference available to them was obtained by measuring the spectra of the noise reaching an ear from different azimuthal locations. Finally, the packing density of a bat's retinal ganglion cells was determined in order to evaluate the relation between the width of the field of best vision and sound-localization acuity.

2. Methods

The behavioral sound-localization tests used a conditioned avoidance procedure in which a hungry animal ate steadily from a food spout while sounds were presented from a loudspeaker to its right, but ceased eating when sounds were presented from a loudspeaker to its left in order to avoid a mild shock (Heffner and Heffner, 1995). An estimate of the interaural intensity differences available to the bats was obtained by measuring the spectra of a broadband noise reaching an ear from a loudspeaker located 30° to the left and 30° to the right of their midline. The anatomical procedure involved mapping the ganglion-cell densities throughout the retina of a big brown bat.

2.1. Subjects

Four wild-caught big brown bats (*E. fuscus*), one female (labelled A) and three males (labelled B, C, and D), were used in the behavioral tests while a fifth bat was used for the anatomical analysis of the retina and a sixth bat was used for the interaural spectral measures. The animals were individually housed with free access to water (supplemented with vitamins) and received a meal worm food paste during the daily test session. The animals typically consumed 8–10 cc of meal worm paste in sessions lasting 40–60 min. Additional supplements of meal worms were given as needed to maintain body weight comparable to that of wild bats.

2.2. Behavioral apparatus

Testing was conducted in a carpeted, double-walled acoustic chamber (IAC model 1204; $2.55 \times 2.75 \times 2.05$ m), the walls and ceiling of which were lined with egg-crate foam. The equipment for behavioral control and stimulus generation was located outside the chamber and the animals were observed via closed-circuit television.

The animals were tested in a cage ($37 \times 22 \times 23$ cm) constructed of 0.5-in (1.26-cm) hardware cloth, mounted 70 cm above the floor on a tripod (see Koay et al., 1997 for an illustration of the test cage). A food spout (2-mm diameter brass tube topped with a 4×6-mm 'lick' plate) was mounted vertically so that it projected up through the bottom of the cage 6 cm above the cage floor. The spout was attached to a 10-cc syringe located below the cage that served as the food reservoir. A meal worm paste consisting of a mixture of 40 meal worms, 1 tablespoon cottage cheese, and 2 tablespoons water, finely blended and sieved through a tea strainer (0.5×1.0-mm openings), was dispensed through the spout by a syringe pump similar to that described elsewhere (Thompson et al., 1990).

During testing, the bats were placed on a small platform ($10 \times 6.5 \times 6.5$ cm) located directly behind the spout. The platform was covered with a 2-mm thick dampened cellulose sponge to facilitate electrical contact. The tip of the food spout was placed in front of and approximately 5 mm below the front of the platform to minimize obstructions between the animal's ears and the loudspeaker. The bat positioned itself above and slightly behind the spout while eating such that the lick plate was entirely covered by its jaw and

could not interfere with the sound field. A contact circuit, connected between the food spout and platform, served to detect when an animal made contact with the spout and activated the syringe pump. Requiring the bat to maintain mouth contact with the spout served to fix its head within the sound field.

Finally, a mild shock was delivered by a shock generator connected between the food spout and platform. The shock was adjusted for each individual to the lowest level that produced a consistent avoidance response to a readily detected signal. The mildness of the shock was indicated by the readiness of the animals to return to the spout after the shock had been delivered. A 25-watt light, mounted 0.5 m below the cage, was turned on whenever the shock was on so that turning off the light indicated that the shock was over and that the animal could return to the spout.

2.3. Acoustical apparatus

Three types of acoustic stimuli were used to assess sound-localization ability: broadband noise bursts, pure tones, and an echolocation call recorded from one of the bats. The sounds were presented through ribbon tweeters (Foster E110T02) mounted on a perimeter bar (102 cm radius, 75 cm height) that was centered on the position occupied by an animal's head while it was eating from the food spout.

2.3.1. Noise

Broadband noise bursts, 100 ms in duration, were generated by a noise generator (Grason-Stadler 1285; set to produce energy up to 100 kHz) and its output was randomly attenuated over a 7-dB range (Coulbourn S85-08 programmable attenuator) from one trial to the next to reduce the possibility of the animals responding on the basis of small intensity differences that may have appeared between the speakers. The signal was then sent to a rise-fall gate (Coulbourn S84-04; 0.1 ms rise/fall), split into left and right channels, amplified to 64-dB sound pressure level (SPL) (Crown D-75 amplifier), and routed to the speakers. Training was carried out using trains of noise bursts (2/s) and final testing was conducted using single 100-ms noise bursts. See Fig. 1 for the spectrum of the noise.

2.3.2. Tones

Sine waves were generated by a tone generator (Krohn-Hite 2400 AM/FM Phase Lock Generator) and randomly attenuated over a 3-dB range (Coulbourn S85-08 programmable attenuator) from one trial to the next. The tones were pulsed, 100 ms on and 500 ms off, for three pulses, shaped by a rise-fall gate (Coulbourn S84-04; 10 ms rise/fall) and bandpass filtered (Krohn-Hite 3202; set 1/3 octave above and below the frequency of the tone). Finally, the signal was split into left and right channels, separately amplified (Crown D-75) and sent to the loudspeakers. The acoustic signal at the location of a listening bat was analyzed for overtones using a spectrum analyzer (Zonic 3525) and any harmonics in the acoustic signal were at least 40 dB below the fundamental and below the animal's threshold.

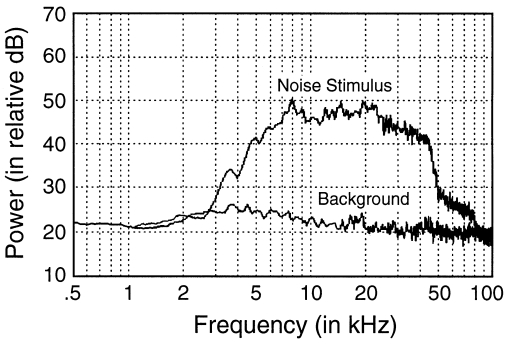

Fig. 1. Spectrum of the broadband noise used for sound localization (upper curve) and spectrum of the background noise (lower curve). The signal was presented at a level of 64-dB SPL. As big brown bats hear from 3.6–105 kHz at a level of 60-dB SPL (Koay et al., 1997), this signal encompassed most of their hearing range.

Testing was conducted in half-octave steps from 5.6 kHz to 64 kHz with the loudspeakers 60° apart (30° to the left and right of midline). The tones were presented at a constant level of 50 dB above the average absolute threshold for the big brown bat (see Koay et al., 1997). Additional testing was conducted by amplitude modulating the 5.6 kHz tone at the rate of 500, 750, and 1000 Hz (100% modulation depth; Krohn-Hite 2400 AM/FM Phase Lock Generator).

2.3.3. Echolocation call

Big brown bats use frequency-modulated sweeps usually less than 2 ms in duration for echolocation. The echolocation calls produced by bat C were recorded in the acoustic chamber using a 1/4-in (6.4-mm) microphone (Brüel and Kjaer 4135), preamplifier (Brüel and Kjaer 2619), measuring amplifier (Brüel and Kjaer 2608), and spectrum analyzer (Zonic 3525). The microphone was held approximately 5 cm in front of the bat while it was scanning its surroundings and the signals were digitized at a sampling rate of 256 kHz and stored in the spectrum analyzer. The call selected for use as a sound-localization stimulus (Fig. 2) was played back by the spectrum analyzer during testing.

For testing, the echolocation call, which was approximately 1.5 ms in duration, was repeated every 16 ms for a total duration of 96 ms (6 repetitions) during each trial. The signal was led from the spectrum analyzer to an attenuator (Coulbourn S85-08), gated on at the beginning of each trial with a rise-fall gate (Coulbourn S84-04, 10 ms rise and fall), split into left and right channels, amplified (Crown D-75), and sent to the loud-

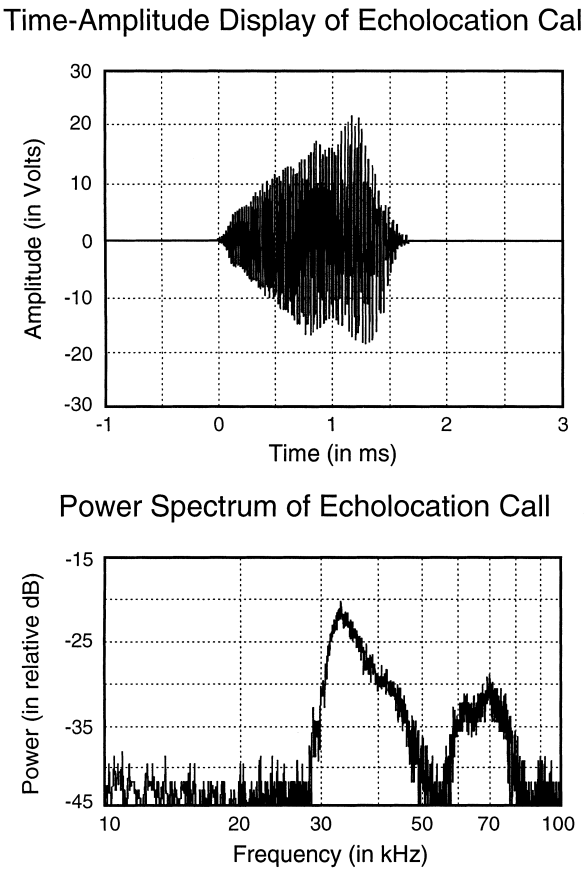

Fig. 2. Time-amplitude display (top) and power spectrum (bottom) of the echolocation call that was used as a localization stimulus. Note that the duration was approximately 1.5 ms and there were two peaks of energy at approximately 34 and 68 kHz.

speakers. The intensity of the train of echo pulses was set to 64-dB SPL, the same level as the noise bursts.

2.3.4. Sound level measurement

The sound pressure levels of the stimuli (SPL re 20 μNewton/m^2) were measured and the left and right loudspeakers equated daily with a 1/4-in (0.64-cm) microphone (Brüel and Kjaer 4135), preamplifier (Brüel and Kjaer 2619), measuring amplifier (Brüel and Kjaer 2608), and filter (Krohn-Hite 3202). The measuring system was calibrated with a pistonphone (Brüel and Kjaer 4230). Sound measurements were taken by placing the microphone in the position occupied by the animal's head and pointing it directly towards a loudspeaker (0° incidence).

2.3.5. Interaural intensity difference

An estimate of the interaural intensity differences available to the big brown bat was obtained for comparison with the results of the tone-localization test. This was accomplished by inserting a 1/8-in microphone (3.2-cm, Brüel and Kjaer 4138) with probe tube through a ventral incision at the base of the concha of a preserved bat. The probe tube was placed so that the tip was located between the entrance to the auditory meatus and the base of the tragus. The signal from the microphone was amplified (Brüel and Kjaer 2169 preamplifier and Brüel and Kjaer 2608 measuring amplifier), filtered (Krohn-Hite 3202, 0.500 kHz to 100 kHz), and fed to a spectrum analyzer (Zonic 3525).

Measurements were taken with the bat placed in the same sound field as used for the behavioral tests. A loudspeaker (Foster E110T02 ribbon tweeter) was placed 30° to the left or right of the animal's midline and 26 cm from the center of its head. The difference in the intensity of a broadband noise at the two ears was then determined for selected frequency bands 1/6 octave in width.

2.4. Behavioral procedure

The animals were first trained to eat steadily from the food spout in the presence of a series of four 100-ms broadband noise bursts (400-ms interburst intervals), presented from a loudspeaker located 90° to the right of the animal. Next, the animals were trained to break contact with the spout (a 'detection response') whenever the noise bursts were presented from a loudspeaker located 90° to their left in order to avoid a mild electric shock delivered via the spout (1.8 s after left signal onset). Breaking contact with the spout usually lasted for the entire trial and indicated that the animal had detected the shift in locus. The light bulb located underneath the cage was turned on while the shock was present at the spout (0.5 s). The light provided feedback for a successful avoidance (since in those cases no shock was actually received by the bat) and permitted the animals to distinguish between successful avoidance of a shock and false alarms (breaking contact when they did not need to since no signal and no shock were presented). After the animals were trained in the basic avoidance procedure, the signals were reduced to one noise burst per trial.

Test sessions consisted of a series of 1.8-s trials that began with the onset of a stimulus. In order to present the trials at a slower pace, the 1.8-s trial intervals were separated by 1.5-s intertrial intervals during which no signals were presented with the result that the animals received one signal every 3.3 s and made a decision after each as to whether to break contact or to continue eating. The response of an animal on each trial (i.e. whether or not it made a detection response) was operationally defined as the duration of contact with the spout during the last 150 ms of each trial which gave the animal sufficient time to react to the signal. If the animal broke contact for more than half of this 150-ms period, a detection response was recorded. The response was classified as a 'hit' if the preceding signal had come from the animal's left side and as a 'false alarm' if it

had come from the animal's right. (Breaking contact during the intertrial interval had no effect on the presentation of trials. However if the bat was not in contact with the spout during the 1 s preceding a trial, data from that trial were not recorded even though the trial proceeded as usual. This avoided using trials when the animal was grooming, or otherwise not participating.)

Each trial had a 22% probability of containing a left signal. The sequence of left-right trials was quasi-random and is described in detail elsewhere (Heffner and Heffner, 1995). Both hit and false alarm *rates* were determined for each block of 8–10 left trials and the associated right trials for each stimulus type and angle. The hit rate was then corrected for the false alarm rate to produce a performance measure according to the formula: Performance = hit rate−(false alarm rate × hit rate). This measure varies from 0 (no hits) to 1 (100% hit rate with no false alarms). Note that the calculation proportionately reduces the hit rate by the false alarm rate observed for each angle rather than the false alarm rate averaged for the session as a whole.

Noise-localization thresholds were determined for a single 100-ms broadband noise burst and for a 100-ms train of echolocation pulses. The angular separation between the left and right loudspeakers was gradually reduced symmetrically around the midline, with blocks of trials containing 8–10 left signal trials given at each angle, until the animal could no longer perform the discrimination (binomial distribution, $P > 0.05$). Daily testing continued until performance no longer improved at any angle. The mean of the best three sessions was then plotted as the asymptotic performance curve for each individual. Threshold was defined as the angle yielding a performance score of 0.50, which was usually determined by interpolation. The actual angles tested were 180°, 120°, 90°, 60°, 45°, 30°, 20°, 15°, 10°, and in one case 5°.

Tone-localization tests were conducted at a fixed angular separation of 60° (30° to the left and right of the animal's midline), with the animal's performance calculated for blocks of trials containing 8–10 left trials. Testing was carried out using a single frequency per session for frequencies that sustained good performance. However, if an animal had difficulty or was unable to localize a particular frequency, as happened at the lowest two frequencies tested, tones of a localizable frequency were presented for several trials to verify that the animal was sufficiently motivated. Each frequency was tested over several non-consecutive sessions for an average of 168 warning trials. The top 25% of the scores were averaged to represent the best performance of which the animals were capable.

2.5. Anatomical procedure

A bat was anesthetized with an overdose of ketamine (80 mg/kg) plus xylazine (4 mg/kg) intramuscularly and perfused with 0.9% saline, followed by 10% formalin. The superior surface of the eyes was marked with fine suture and the eyes were removed and the retinae dissected free from the sclera. The retinae were then mounted on gelatinized slides with the ganglion-cell layer uppermost, and stained with thionine (Stone, 1981). The density of the ganglion cells was determined throughout the retina in 0.1-mm steps through the horizon of the retina and in 0.2–0.3-mm steps in the superior and inferior periphery. The number of ganglion-cell nucleoli within a sampling rectangle 37 × 25 μm (0.000925 mm^2) were counted using a 100 × oil immersion objective. The horizontal width of the region encompassing ganglion-cell densities equal to or greater than 75% of maximum density was determined as an indication of the width of the field of best vision for comparison between species. The maximum number of cells/$degree^2$ was used to calculate the maximum theoretical resolvable spatial frequency in cycles per degree (i.e. the maximum number of cycles of a square wave grating–alternating black and white bars that can be resolved per degree of visual angle) using Shannon's sampling theorem (e.g. DeBruyn et al., 1980). For additional details of the method see Heffner and Heffner (1992b).

These experiments were carried out with the approval of the University of Toledo Animal Care and Use Committee.

3. Results

3.1. Noise localization

The ability of the three big brown bats to discrim-

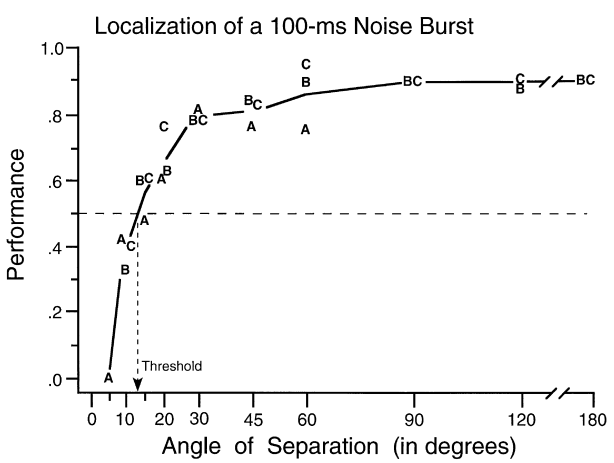

Fig. 3. Sound-localization performance of three big brown bats for the 100-ms noise burst. Letters represent individual animals and the horizontal dashed line indicates the 0.50 performance level used to define threshold. Chance performance was approximately 0.40 in this task.

inate 100-ms noise bursts emitted from loudspeakers centered symmetrically about midline is illustrated in Fig. 3. The animals performed reliably at large angles, achieving average performances of 0.80 or better with performances rapidly declining as the angle of separation fell below 30°. The thresholds (minimum audible angles) for animals A, B, and C were 16°, 12°, and 13° respectively for an overall average of 14°. The close agreement between the three animals suggests that the thresholds obtained are representative for big brown bats.

3.2. Localization of an echolocation call

The ability of bat C to passively localize one of its own echolocation calls is shown in Fig. 4. As can be seen, the animal's performance in localizing playbacks of its own call parallels its performance in the noise-localization task. Localization threshold for the call was 15°, which is within the range of the thresholds for localizing noise and very close to its own 13° noise-localization threshold. Thus, there is no noticeable difference in thresholds for passively localizing a noise burst or an echolocation call.

3.3. Pure-tone localization

To determine the ability of the big brown bat to use binaural time- and intensity-difference cues to localize sound, two animals were tested for their ability to localize brief tone-pips ranging in frequency from 5.6 kHz to 64 kHz. This test is based on the absence of binaural intensity-difference cues at low frequencies because low frequencies bend around the head with little or no attenuation. On the other hand, binaural time cues, in the form of the phase-difference cue, become ambiguous at high frequencies. At a 60° angle of separation between sound sources, the calculated frequency above which the phase cue becomes physically ambiguous for an adult big brown bat with an interaural distance of 55 μs is 21 kHz (for the formula for calculating the frequency of ambiguity, see Kuhn, 1977). Thus, animals that use both binaural cues are able to localize both low and high frequencies, although they often show a dip in performance in the midrange where neither cue is maximally effective (e.g. Heffner and Heffner, 1992a; Masterton et al., 1975). Animals that lack the ability to use the binaural phase cue are unable to localize low frequencies whereas those that cannot use binaural intensity differences are unable to localize high frequencies.

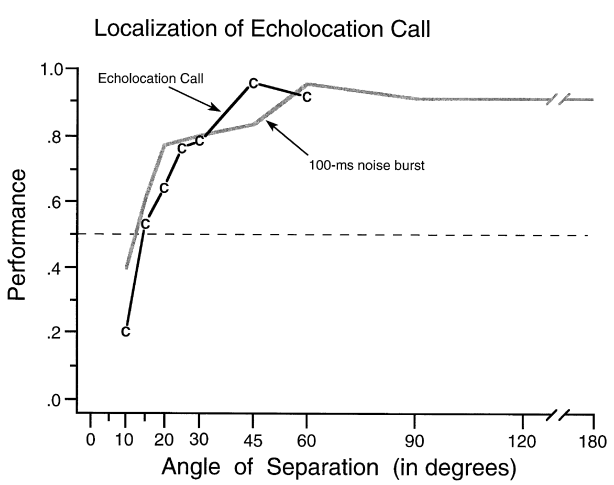

Fig. 4. Sound-localization performance of bat C for a train of six echolocation calls (C). Shaded line indicates performance of the same bat localizing a single 100-ms burst of noise (taken from Fig. 3). Note that the two performances are not noticeably different.

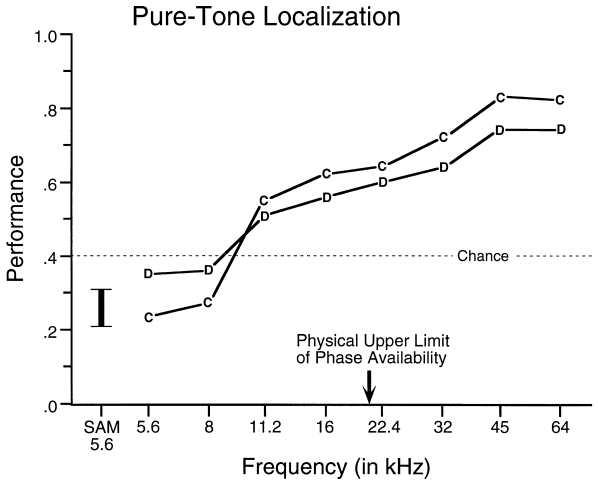

Fig. 5. Sound-localization performance of two big brown bats as a function of the frequency of a pure-tone stimulus for a fixed angle of separation (±30° azimuth). Letters represent individual animals; arrow indicates the upper limit of the physical availability of the binaural phase-difference cue for the big brown bat. Note that performance falls to chance at frequencies below 11.2 kHz and does not improve when the 5.6-kHz signal is sinusoidally amplitude modulated (SAM) at 500, 750, or 1000 Hz. Vertical bar indicates the range of scores of the two bats for the amplitude-modulated signals.

The performances of the two bats on this test indicate that the big brown bat can use binaural intensity, but not binaural time cues. As shown in Fig. 5, the performance of each bat was quite good at 45 and 64 kHz, the highest frequencies tested, but declined steadily with decreasing frequency, falling to chance at 8 and 5.6 kHz, the lowest frequencies tested. This pattern of performance is typical of an animal that lacks the ability to use the binaural time-difference cue (cf. Masterton et al., 1975). That is, performance is good at high frequencies where binaural intensity differences are maximal, declines with frequency as the head and pinnae are becoming less effective in attenuating the sound, and finally falls to chance at low frequencies where the binaural intensity differences approach zero.

During testing, it occurred to us that the bats might be capable of using binaural time cues, but that their

Table 1
Performance of bats C and D in discriminating the locus of a 5.6-kHz tone that was amplitude modulated at three modulation rates

Modulation rate	Performance scores	
	Bat C	Bat D
Unmodulated	0.24	0.36
500 Hz	0.30	0.31
750 Hz	0.22	0.30
1000 Hz	0.21	0.23

Loudspeakers were located at ±30° azimuth. Note that modulating the 5.6-kHz tone did not improve performance and that none of the scores were above chance ($P > 0.05$).

use of binaural phase was limited for physiological reasons to frequencies below 5.6 kHz. However, because the absolute sensitivity of the bats declines rapidly below 8 kHz, it was not possible to present frequencies lower than 5.6 kHz at sufficient intensities without noticeable distortion. To circumvent this problem, we tested the animals with a 5.6-kHz tone that was sinusoidally amplitude modulated at rates of 500, 750, or 1000 Hz to provide them with binaural time cues in the form of the envelope of the modulated tone. However, once again, both animals failed to perform above chance at any of the amplitude modulation rates, thereby indicating that they could not use the binaural time cues provided by the envelope of the signal (Fig. 5 and Table 1). In short, we found no evidence that the big brown bat could use binaural time-difference cues.

3.4. Interaural intensity difference

The decline in interaural intensity differences as frequency decreases was demonstrated by measuring the spectra of a broadband noise reaching one ear for loudspeaker locations of 30° to the left and right of midline. As can be seen in Fig. 6, interaural intensity differences are approximately 10 dB at 32 kHz and decline steadily

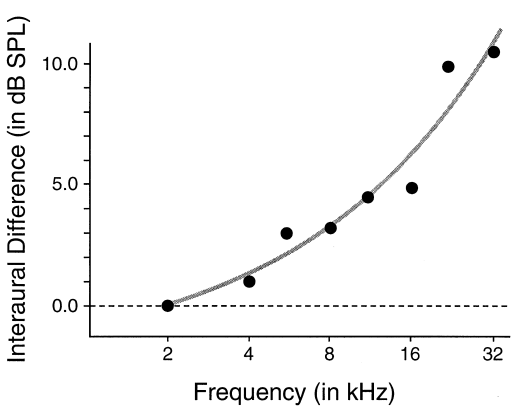

Fig. 6. Interaural intensity differences across frequency as determined by measuring the spectra of a broadband noise reaching one ear for loudspeaker locations of 30° to the left and 30° to the right of midline (0° elevation).

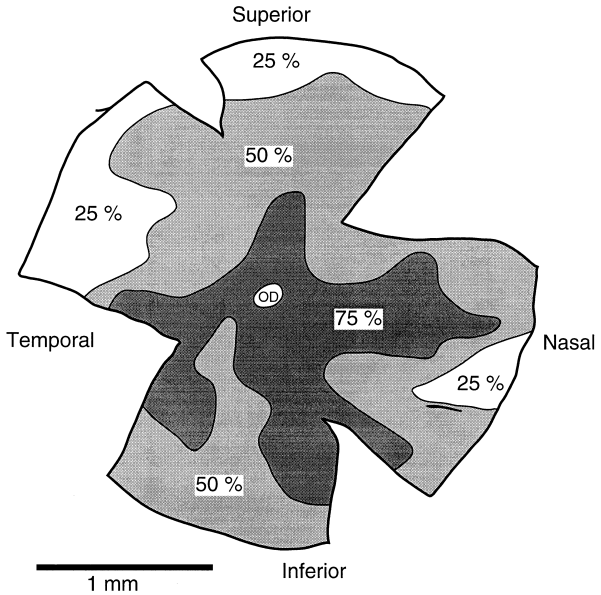

Fig. 7. Retinal ganglion-cell isodensity contours in the right retina of a big brown bat. Density is expressed as a proportion of the maximum density (8649 cells/mm^2); dark grey indicates densities at least 75% of maximum, light grey indicates densities at least 50% of maximum, and white indicates densities at least 25% of maximum (minimum densities did not fall below 25% of maximum). OD, optic disk.

to 0 dB by 2 kHz. At 8 kHz, the frequency at which the bats' pure-tone-localization performance fell to chance (cf. Fig. 5), the interaural intensity difference was approximately 3 dB. These results show good agreement with those of Jen and Chen (1988) who obtained differences in big brown bats of approximately 7 dB for frequencies as low as 25 kHz (lower frequencies not tested). Measures in a similar-sized mouse also produced differences of 3 dB or less at frequencies below 10 kHz (Chen et al., 1995). Thus, the decline in pure-tone-localization performance by big brown bats as frequency decreases is accompanied by a corresponding decrease in the availability of interaural intensity differences.

3.5. Retinal ganglion-cell densities

The retina of the big brown bat is avascular and approximately 2.9 mm in diameter. The ganglion cells are relatively small (5–10-μm diameter) with large eccentric nuclei and usually a clearly distinguishable nucleolus. The ganglion cells reach a relatively high peak density of 8649 cells/mm^2 in small regions of both the nasal and temporal retina. This density approximates that found in cats, but owing to the very small size of the eye, it corresponds to a maximum theoretical acuity of only 0.75 cycles/degree. This estimate of acuity agrees well with the 0.5 cycles/degree determined by the optomotor response (Bell and Fenton, 1986) and the 0.7 cycles/degree obtained by a previous analysis

of the retinal ganglion-cell density (Marks, 1980). The visual acuity of the big brown bat is thus less than the 60 cycles/degree of humans, the 9 cycles/degree acuity of cats, and the ~1.5 cycles/degree acuity of domestic rats, and is on par with the 0.4 cycles/degree estimated for subterranean mole rats (Birch and Jacobs, 1979; Heffner and Heffner, 1993; Hughes, 1977; Jacobson et al., 1976). Compared with other bats, the visual acuity of big brown bats, like that of other nocturnal insectivorous bats, is relatively poor as opposed to the visual acuity of crepuscular insectivorous and nocturnal fruit-eating bats (e.g. Bell and Fenton, 1986; Pettigrew et al., 1988).

The density of the ganglion cells falls irregularly toward the periphery as illustrated in Fig. 7, but remains greater than 50% of peak density nasally and greater than 25% of peak density temporally. Such shallow density gradients are typical of microchiropteran bats (Pettigrew et al., 1988) and the irregular isodensity contours we observed confirm the observations of others for the big brown bat (Marks, 1980).

The isodensity contour demarcating densities at least 75% of maximum reveals an irregular visual streak passing through the optic disk and encompassing 139° of the horizon. As discussed below, the association of this relatively broad field of best vision with relatively poor passive sound-localization acuity corresponds to the pattern seen in non-echolocating mammals.

4. Discussion

4.1. Passive localization acuity of big brown bats compared with other mammals

Although no previous measure of the passive sound-localization ability of the big brown bat exits, an indirect estimate based on echolocation acuity has been offered by Simmons and his colleagues (Simmons et al., 1983). Based on the observation that the big brown bat can discriminate differences in spacing between pairs of vertical rods with an acuity of 1.5°, which they believed to be mediated by a binaural mechanism, they suggested that the passive localization acuity of big brown bats might also be 1.5°. As the present results show, their actual passive localization threshold, 14°, is much larger. This difference suggests that active and passive localizations are distinct abilities relying on different mechanisms. Indeed, a similar distinction between active and passive localization has been proposed for the little brown bat (*Myotis lucifugus*) on the basis of midbrain responses that suggest that different mechanisms may be used in the neural analysis underlying the two types of localization (Condon et al., 1996). The potential mechanisms are broad and remain to be identified, however it should be noted that a distinction between active and passive perception has precedent in another sensory system: somatosensation (Hutson and Masterton, 1986; Semmes, 1973).

Compared with other mammals, the 14° threshold of the big brown bat is not unusual. As shown in Fig. 8, it lies in the midrange of those species tested so far. Moreover, the big brown bat conforms to the general mammalian plan in which species with narrow fields of best vision have better sound-localization acuity than those with broader fields, a relationship described in detail in Section 4.3.

One species of bat for which a passive sound-localization threshold is available is the pallid bat, *Antrozous pallidus*. Using a procedure in which a bat flew from a fixed perch to an anesthetized cricket tossed onto the floor, Fuzessery et al. (1993) estimated the species' passive localization threshold to be ±1°. Such acuity is rare among mammals and would make the pallid bat one of the most acute mammals yet tested (cf. Fig. 8), placing its acuity very near that of humans (Mills, 1958). Although surprising, such acuity could be a result of the pallid bat's reliance on passive localization for prey capture, relegating echolocation primarily to navigation (Fuzessery et al., 1993). However, the remarkable acuity of the pallid bat raises questions such as why it requires better localization acuity to hunt its prey than do other small carnivorous mammals (e.g. the least weasel, Fig. 8), what mechanisms it uses to achieve such accuracy, and whether it fits the relationship between size of the field of best vision and sound-localization acuity seen in other mammals. Thus, it would be of interest to confirm the pallid bat's threshold using standard psychophysical procedures.

4.2. Use of binaural locus cues

The ability to localize pure tones has been used to demonstrate the duplex theory of sound localization in which the ability to localize low frequencies is attributed to the use of the binaural time- or phase-difference cue and the ability to localize high frequencies is attributed to the use of the binaural intensity-difference cue (Stevens and Newman, 1936). Although most mammals, including humans, monkeys, cats, and rats, are able to use both binaural locus cues, it has become apparent that others lack the ability to use one or the other, or even both cues (Heffner and Heffner, 1992a). Specifically, the hedgehog and spiny mouse do not use binaural time cues, the Indian elephant, horse, cattle, domestic goat, and domestic pig lack the ability to use binaural intensity cues over part or all of their high-frequency hearing range, and the pocket gopher, blind mole rat, and naked mole rat lack the ability to localize sound using either cue (Heffner and Heffner, 1990, Heffner and Heffner, 1992a, Heffner and Heffner, 1992c, Heffner and Heffner, 1993).

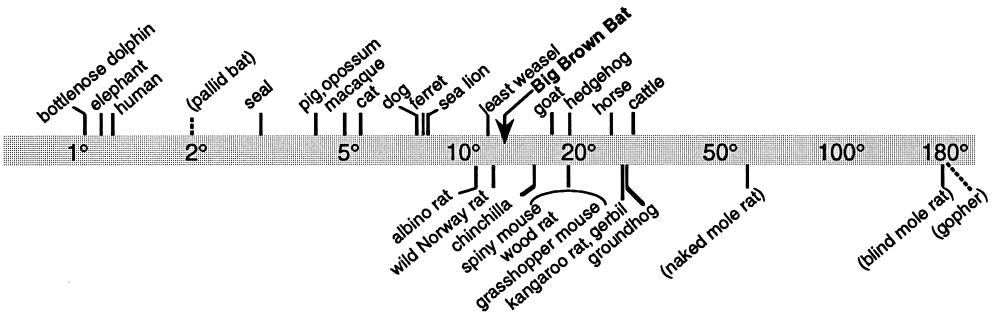

Fig. 8. Azimuthal sound-localization acuity for 29 species of mammals. All thresholds were obtained using stimuli of 100 ms or less with the exception of the subterranean species and pallid bat, indicated by parentheses. The subterranean species required longer duration sounds before they could successfully localize and, even then, the gopher could barely localize above chance. For references, see Heffner et al. (1994).

The results reported here indicate that the big brown bat can use the binaural intensity-difference cue, but not the binaural phase-difference cue, to localize sound. That is, the bats were able to localize pure tones from 11.2 to 64 kHz, indicating that they could use the binaural intensity cue, but they were unable to localize 5.6 and 8 kHz, suggesting that they could not use the binaural phase cue. The interaural intensity differences available to big brown bats for sources located at 0° elevation and 30° to the left or right of midline were at least 10 dB for frequencies above 22.8 kHz, but fell to 5 dB at 16 kHz and continued falling to 3 dB or less at 8 kHz and below — frequencies that the bat could not localize. In the absence of the ability to use the interaural phase cue, the animals were left with a declining interaural intensity difference (Fig. 6) on which to base their localization judgements and their performance fell accordingly as frequency decreased.

Because the poor low-frequency sensitivity of the big brown bat makes it difficult to generate lower frequencies at sufficient intensities without noticeable distortion, the animals were further tested with a 5.6-kHz tone that was amplitude modulated at 0.500, 0.750 and 1 kHz. Such a signal provides time or phase information in its envelope. Specifically, it has been shown that humans can lateralize a high-frequency signal when it is modulated at a low frequency (e.g. McFadden and Pasanen, 1976). Unlike humans, however, the bats were unable to localize the amplitude-modulated tone. We interpret this result to indicate that the big brown bat cannot use the binaural phase cue down to at least 500 Hz and, therefore, is probably incapable of using binaural time cues at all. Since the hearing range of the big brown bat extends only down to about 3.7 kHz (Koay et al., 1997), its hearing does not include the frequencies below 3 kHz where phase locking occurs in other mammals (cf. Johnson, 1980). From our results it seems that, despite their other auditory specializations, big brown bats have not developed an auditory system capable of phase locking to high frequencies even though that is not beyond the capacity of a vertebrate brain, as demonstrated by barn owls (Sullivan and Konishi, 1984).

Because previous studies have indicated that the ability to use binaural locus cues is reflected in the morphology of the auditory brainstem (e.g. Masterton et al., 1975; Heffner and Heffner, 1992a), the question arises as to the morphology of the big brown bat's brainstem nuclei. In the big brown bat, the lateral superior olivary nucleus, which receives high-frequency input from the two ears and is believed to mediate the binaural intensity-difference cue, is well developed, as are the intermediate and ventral nuclei of the lateral lemniscus, which receive monaural input. However, the medial superior olivary nucleus, which in non-echolocating mammals receives low-frequency input from the two ears and is believed to mediate interaural time differences, is very small in the big brown bat (Casseday and Covey, 1987; Huffman and Covey, 1995). In addition, unlike the common mammalian pattern of strong excitatory input from both cochlear nuclei, the medial superior olive in big brown bats receives largely monaural input, with both excitatory and inhibitory input arising from the contralateral ear (e.g. Kuwabara and Zook, 1992). Although it has been proposed that timing could be accomplished by comparing the excitatory input from one ear with the small inhibitory input from the other (Grothe et al., 1994), this has not yet been demonstrated. Thus, the inability of the big brown bat to use binaural time cues is supported by the lack of evidence for processing those cues in the auditory brainstem.

With the addition of the big brown bat, there are now three species of mammals that appear to be unable to use binaural time cues for localizing sound: the hedgehog (*Paraechinus hypomelas*, Masterton et al., 1975), spiny mouse (*Acomys cahirinus*, Heffner and Heffner, 1992a), and now the big brown bat. One feature these animals have in common is that they have relatively small heads or, more specifically, small interaural distances. The time it takes for sound to travel around the head from one auditory meatus to the other

is 55 μs, 64 μs, and 167 μs for the big brown bat, spiny mouse, and hedgehog, respectively. Because the magnitude of the binaural time cue for a given angle is dependent on head size, animals with very small heads might relinquish the use of the binaural time cue because their heads are too small to generate useful time differences. However, while this explanation may account for the inability of the big brown bat and the spiny mouse to use binaural time cues, it does not account for the hedgehog, as there are a number of species of mammals with interaural distances smaller than the hedgehog, but larger than the big brown bat and the spiny mouse, that are able to use binaural time cues; for example, the least weasel (76 μs), gerbil (87 μs), and kangaroo rat (90 μs) (Heffner and Heffner, 1987, 1988; Heffner and Masterton, 1980). Thus, although the small interaural distances of the big brown bat and spiny mouse may have rendered binaural time cues too small to be useful, the same cannot be said for the hedgehog.

4.3. Role of vision in sound localization

We have previously proposed that a major selective pressure influencing the variation in sound-localization acuity among mammals is the need to direct the field of best vision toward a sound source for further scrutiny (Heffner and Heffner, 1992b). Just how accurate sound localization must be to direct the eyes seems to depend on the width of an animal's field of best vision: Animals with narrow fields of best vision, such as humans, require good sound-localization acuity in order to direct their gaze so that the visual image of the sound source falls upon their fovea, whereas animals with broad fields of best vision, such as those with visual streaks, do not require such acuity.

However, exceptions to this relationship are known to occur — specifically, among subterranean animals adapted to living in dark burrows where visual scrutiny of sound sources is not possible. Not only is their vision reduced, but such animals usually have reduced high-frequency hearing and little or no ability to localize sound. Thus, the results of studies on the pocket gopher, blind mole rat, and naked mole rat support the view that vision and sound-localization ability are closely linked by demonstrating that animals that do not use vision also relinquish the ability to localize sound, apparently because they no longer need it to direct their gaze (Heffner and Heffner, 1990, Heffner and Heffner, 1992a, Heffner and Heffner, 1993). Like some of these subterranean species, big brown bats also have small eyes, but unlike the subterraneans, they do forage in the light of dawn and dusk and often roost in places such as eaves of buildings that admit light. Moreover, they orient to the sunset glow (Buchler and Childs, 1982) and have retained measurable vision, although it is relatively poor even compared to that of other bats (Pettigrew et al., 1988; Suthers and Wallis, 1970). Because big brown bats have reduced vision and rely on echolocation instead of vision for navigation and the pursuit of prey, it occurred to us that they, like subterranean mammals, might prove to be unusual compared to other mammals. However, this turned out not to be the case.

The relationship between the width of the field of best vision and passive sound-localization acuity is illustrated in Fig. 9. As can be seen, animals with narrow fields of best vision, such as humans, are more accurate localizers than animals with broader fields of best vision, such as cattle. Among the 23 species of mammals for which data are available, the correlation is very strong and reliable ($r = 0.922$, $P \leq 0.0001$), accounting for 85% of the variance. Moreover, despite its reliance on echolocation, the big brown bat does not deviate significantly from the mammalian pattern as its passive sound-localization threshold falls close to the value predicted by the width of its field of best vision ($t = -1.34$,

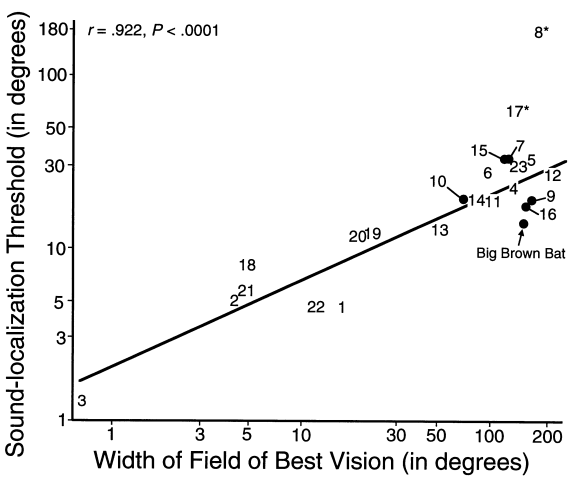

Fig. 9. Relationship between width of the field of best vision (defined by ganglion-cell densities of at least 75% of maximum) and sound-localization threshold for 23 species of mammals. Species with narrow fields of best vision have better localization acuity than species with broad fields of best vision. The big brown bat does not deviate significantly from the regression line ($t = -1.34$, $P = 0.21$). Key: 1, Virginia opossum (*Didelphis virginiana*); 2, Japanese macaque (*Macaca fuscata*); 3, human (*Homo sapiens*); 4, domestic rabbit (*Oryctolagus cuniculus*); 5, eastern chipmunk (*Tamias striatus*); 6, groundhog (*Marmota monax*); 7, black-tailed prairie dog (*Cynomys ludovicianus*); 8, pocket gopher (*Geomys bursarius*); 9, wood rat (*Neotoma floridana*); 10, grasshopper mouse (*Onychomys leucogaster*); 11, hamster (*Mesocricetus auritus*); 12, gerbil (*Meriones unguiculatus*); 13, wild Norway rat (*Rattus norvegicus*); 14, spiny mouse (*Acomys cahirinus*); 15, domestic mouse (*Mus musculus*); 16, chinchilla (*Chinchilla laniger*); 17, naked mole rat (*Heterocephalus glaber*); 18, dog (*Canis familiaris*); 19, least weasel (*Mustela nivalis*); 20, ferret (*Mustela putorius*); 21, cat (*Felis domesticus*); 22, pig (*Sus scrofa*); 23, cow (*Bos taurus*). The two subterranean species (indicated by *) do deviate significantly from the regression line and their inclusion lowers the correlation to 0.855. See Heffner et al. (1994) for references.

$P = 0.21$). The poor visual acuity of the big brown bat is not likely a factor affecting its sound-localization acuity; it appears that mammals use sound localization to direct their best vision to the source of a sound regardless of the absolute acuity of that vision (Heffner and Heffner, 1992b).

It is not impossible, however, that the passive sound-localization acuity of the big brown bat might be linked to its echolocation ability. For example, some bats might conceivably use passive sound localization to direct their echolocation signal instead of, or in addition to, directing their best vision. If so, then one might expect sound-localization acuity to vary as a function of the width of the echolocation beam. However, there is insufficient information on beam width and passive sound-localization acuity of bats to test this hypothesis and no independent evidence that bats coordinate their passive localization with echolocation. Thus, it would appear at this time that the eyes have it and that big brown bats, like other mammals, use their passive sound localization to direct their best vision to the source of a sound.

Acknowledgments

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