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

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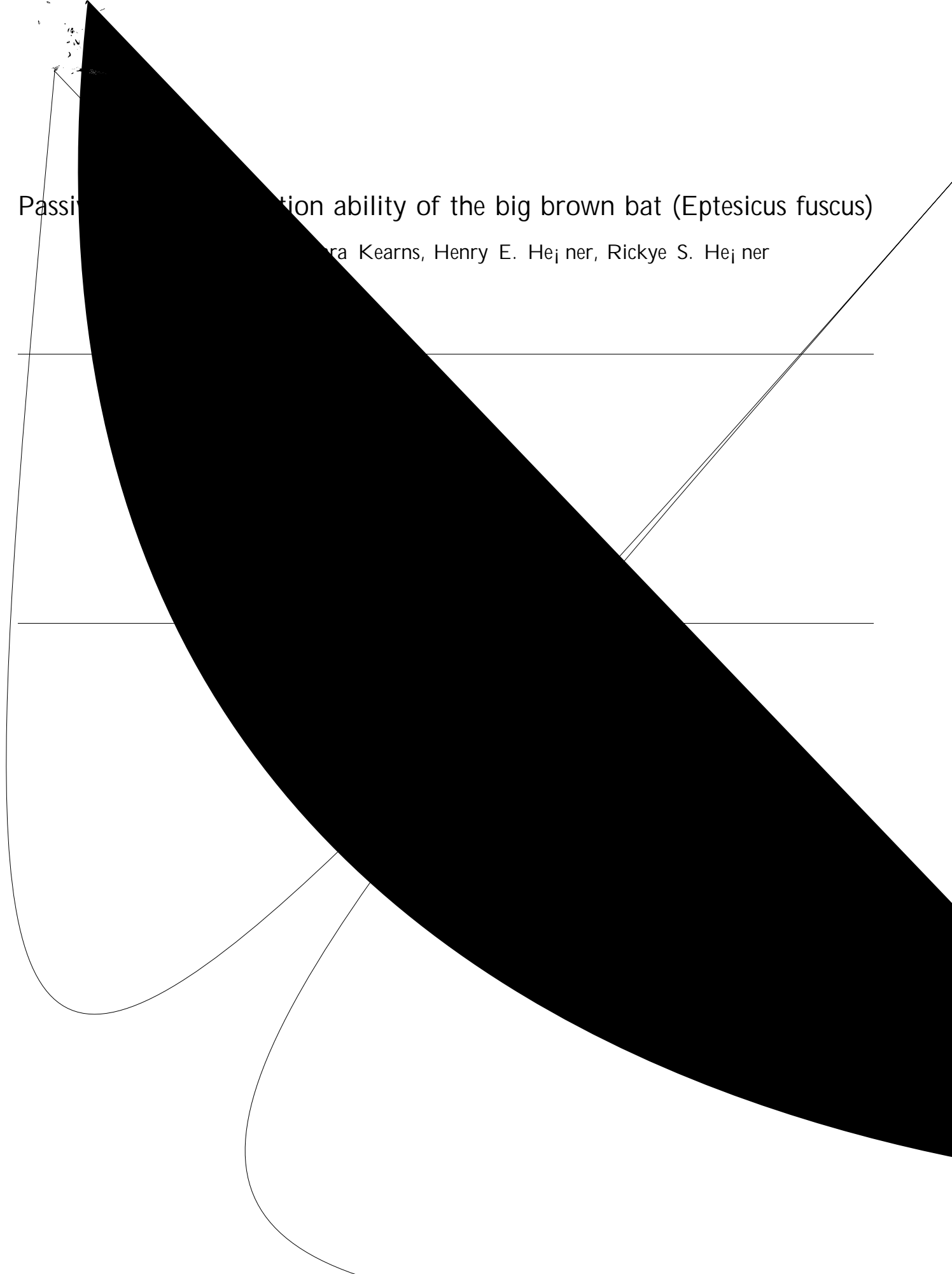
are loudest and, presumably, concentrations of prey are the greatest (Buchler and Childs, 1981). However, such studies cannot tell us how accurate the bats are at localizing sound nor what sound-localization cues they use.

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. Mᵣ҂ ҆

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 differ-

could not interfere with the sound ¢eld. 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 ¢x its head within the sound ¢eld.

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 o¡ 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 di¡erences 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, ampli¢ed to 64-dB sound pressure level (SPL) (Crown D-75 ampli¢er), and routed to the speakers. Training was carried out using trains of noise bursts (2/s) and ¢nal 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 o¡, for three pulses, shaped by a rise-fall gate (Coulbourn S84-04; 10 ms rise/fall) and bandpass ¢ltered (Krohn-Hite 3202; set 1/3 octave above and below the frequency of the tone). Finally, the signal was split into
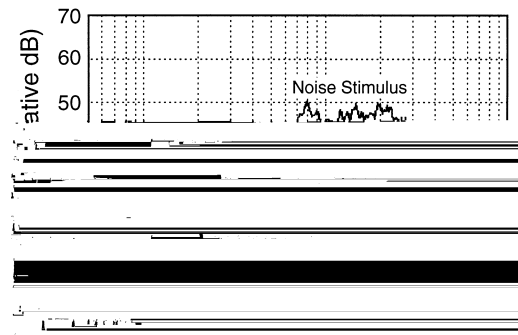

Fig. 1. Spectrum of the broadband noise used for sound localization (upper curve) and spectrum of the background noise (lower curve). The signal was presented at a level of 64-dB SPL. As big brown bats hear from 3.6^105 kHz at a level of 60-dB SPL (Koay et al., 1997), this signal encompassed most of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

left and right channels, separately ampli¢ed (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.

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 (Brûel and Kjaer 4135), preampli¢er (Brûel and Kjaer 2619), measuring ampli¢er (Brûel 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, ampli¢ed (Crown D-75), and sent to the loud-

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), preampli¢er (Brüel and Kjaer 2619), measuring ampli¢er (Brüel and Kjaer 2608), and ¢lter (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 di¡erence

An estimate of the interaural intensity di¡erences 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 pre-served 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 ampli¢ed (Brüel and Kjaer 2169 pre-ampli¢er and Brü2burKj5645 -4Kj559bigou4aeished3.2-37-loudoi5

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-di¡erence 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-di¡erence 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-di¡erence cue, become ambiguous at
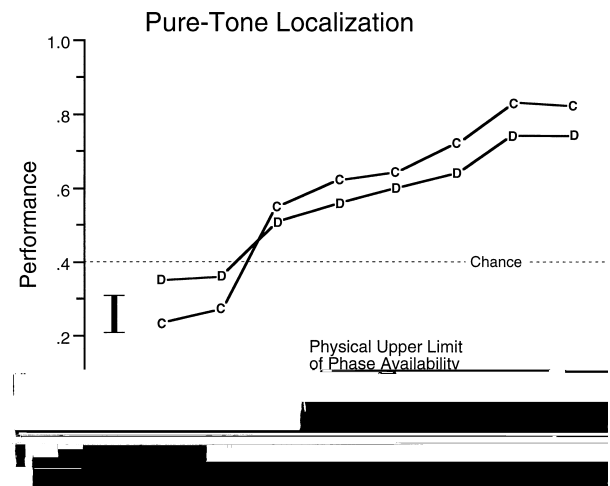
Fig. 5. Sound-localization performance of two big brown bats as a function of the frequency of a pure-tone stimulus for a ¢xed angle of separation (þ 30‡ azimuth). Letters represent individual animals; arrow indicates the upper limit of the physical availability of the binaural phase-di¡erence cue for 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.

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 e¡ective (e.g. He¡ner and He¡ner, 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 di¡erences are unable to localize high frequencies.

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-di¡erence cue (cf. Masterton et al., 1975). That is, performance is good at high frequencies where binaural intensity di¡erences are maximal, declines with frequency as the head and pinnae are becoming less e¡ective in attenuating the sound, and ¢nally falls to chance at low frequencies where the binaural intensity di¡erences approach zero.

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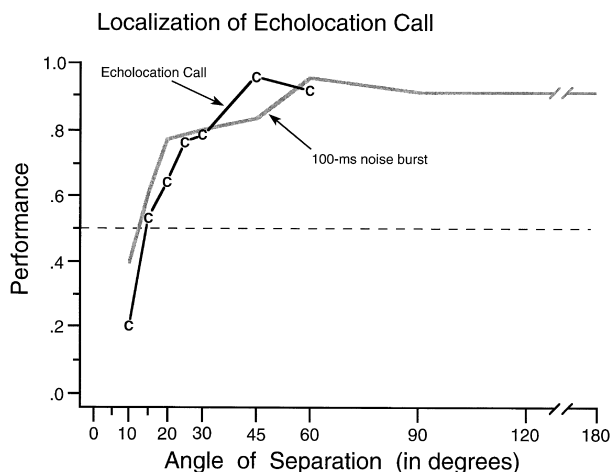

Fig. 4. Sound-localization performance of bat C for a train of six echolocation calls (C). Shaded line indicates performance of the same bat localizing a single 100-ms burst of noise (taken from Fig. 3). Note that the two performances are not noticeably di¡erent.

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; He¡ ner and He¡ ner, 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 con¢rm the observations of others for the big brown bat (Marks, 1980).

The isodensity contour demarcating densities at least

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 inter-

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 di¡erences. 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

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.

Masterton, R.B., Thompson, G.C., Bechtold, J.K., RoBards, M.J., 1975. Neuroanatomical basis of binaural phase-di¡erence analysis for sound localization: A comparative study. J. Comp. Physiol. Psychol. 89, 379^386.

McFadden, D., Pasanen, E.G., 1976. Lateralization at high frequencies based on interaural time di¡erences. J. Acoust. Soc. Am. 59, 634^639.

Mills, A.W., 1958. On the minimum audible angle. J. Acoust. Soc. Am. 30, 237^246.