

Sound localization in common vampire bats: Acuity and use of the binaural time cue by a small mammal

Ricky S. Heffner,^{a)} Gimseong Koay, and Henry E. Heffner

Department of Psychology #948, University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606

(Received 25 May 2014; revised 11 November 2014; accepted 18 November 2014)

Passive sound-localization acuity and the ability to use binaural time and intensity cues were determined for the common vampire bat (*Desmodus rotundus*). The bats were tested using a conditioned suppression/avoidance procedure in which they drank defibrinated blood from a spout in the presence of sounds from their right, but stopped drinking (i.e., broke contact with the spout) whenever a sound came from their left, thereby avoiding a mild shock. The mean minimum audible angle for three bats for a 100-ms noise burst was 13.1°—within the range of thresholds for other bats and near the mean for mammals. Common vampire bats readily localized pure tones of 20 kHz and higher, indicating they could use interaural intensity-differences. They could also localize pure tones of 5 kHz and lower, thereby demonstrating the use of interaural time-differences, despite their very small maximum interaural distance of 60 μ s. A comparison of the use of locus cues among mammals suggests several implications for the evolution of sound localization and its underlying anatomical and physiological mechanisms. © 2015 Acoustical Society of America.

[<http://dx.doi.org/10.1121/1.4904529>]

[ANP]

Pages: 42–52

I. INTRODUCTION

The ability of mammals to localize sound is not uniform, but varies between species in two important ways. First, sound-localization acuity as measured by the minimum audible angle for brief sound ranges from about 1° for humans and elephants to more than 25° in mice, rabbits, and cattle, with subterranean rodents being virtually unable to localize brief sounds at all (Heffner and Heffner, 2003). This variation can be accounted for by the relationship between auditory localization and vision in which species with narrow fields of best vision appear to require more acute locus information to direct their gaze to the source of a sound than do species with broad visual fields or visual streaks (Heffner and Heffner, 1992c).

The second way in which the sound-localization ability of mammals varies is in the use of the binaural time and intensity cues for locus, which are demonstrated by the ability of an animal to localize low- and high-frequency pure tones (e.g., Heffner and Heffner, 2003). Specifically, low-frequency pure tones that bend around the head with little or no attenuation are localized by comparing the time of arrival of the phase of each cycle of the tone at the two ears and this is thus often referred to as the binaural phase-difference cue. The phase-difference cue becomes ambiguous for pure tones at higher frequencies when successive cycles arrive too quickly for the nervous system to match the arrival of the same cycle at the two ears. The exact “frequency of ambiguity” depends on an animal’s head size and the angle of the sound source relative to its midline—it is higher for smaller heads and sound sources closer to midline. Pure

tones above the frequency of ambiguity, then, must be localized using interaural intensity differences if they are available.

Using the ability to localize low- and high-frequency pure tones as an indication of the ability to use the binaural time and intensity cues, it has been found that although most mammals use both binaural cues, many do not. For example, it is now known that hedgehogs, rats, and some bats do not use binaural time cues, whereas pigs, horses, cattle, and alpacas do not use binaural intensity cues (Heffner and Heffner, 1986, 1989; Heffner and Heffner, 2003; Heffner *et al.*, 2014). Subterranean rodents appear to use neither binaural cue, as they are virtually incapable of localizing brief sounds (Heffner and Heffner, 1990, 1992b, 1993). Moreover, the highest-frequency pure tone that can be localized using the interaural phase-difference cue also varies considerably, from 250 Hz for the Indian elephant to 6.3 kHz for the Jamaican fruit bat (Heffner and Heffner, 1982; Heffner *et al.*, 2001c). Why some animals fail to use one or the other binaural locus cue and why the upper frequency limit for the binaural phase cue varies is not currently known.

To further explore this variation in mammalian sound localization, the ability of the common vampire bat (*Desmodus rotundus*) to localize sound was determined. Common vampire bats are best known for their highly specialized digestive physiology and behavior that enables them to live on blood, primarily of large bovids. However, their hearing abilities are also of interest because they are more sensitive to low frequencies than any of the bats tested so far (Heffner *et al.*, 2013). With such relatively good low-frequency sensitivity for a small bat, their performance on a task requiring the use of the low-frequency-based binaural phase cue, despite the small magnitude of the available time difference, was of special interest.

^{a)}Author to whom correspondence should be addressed. Electronic mail: Ricky.Heffner@utoledo.edu

II. METHODS

Sound-localization thresholds (i.e., minimum audible angles) were obtained using the conditioned suppression/avoidance procedure in which a bat was trained to break contact with a reward spout if a brief noise burst was presented from its left side, and to continue feeding from the spout if the noise came from its right. The ability to localize pure tones at an angle of 60° separation was used to determine the ability to use the binaural locus cues.

A. Subjects

Three male *Desmodus rotundus* (referred to as A, B, and C) were approximately 3–3.5 years old at testing and weighed 23–29 g. They were individually housed in wood and plastic mesh cages (48 × 39 × 95 cm). They had free access to water and received their meals of defibrinated blood (Barnard, 1995) during the test sessions. Supplemental feedings were provided as needed to maintain healthy body weight. Blood was collected from local cattle at slaughter time and each gallon was immediately mixed with 11 g sodium citrate, 4 g citric acid, and 12.5 g dextrose. The blood was strained and frozen in 100-ml quantities and stored up to 3 months; thawed blood was refrigerated and discarded after two days. Animal use was approved by the University of Toledo Animal Care and Use Committee.

B. Behavioral apparatus

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

The test cage (28 × 21 × 24 cm) was constructed of 1.5-cm wire mesh, and sat on a tripod 92 cm above the floor (for a drawing of the test cage, see Koay *et al.*, 2002a). The reward spout consisted of a 3-mm-diameter brass tube topped with a brass bowl (8-mm diameter × 5-mm deep). It was attached with silicone tubing to a 10-cc plastic syringe that contained the blood reward. The blood was dispensed using a syringe pump (Yale Apparatus YA-12) housed in a high-density particleboard box (40 × 30 × 30 cm) and lined with egg-crate foam to eliminate pump noise. The box was placed on the carpeted floor behind the cage.

During testing, a bat climbed onto a small platform (15 × 8 × 6 cm) and approached the reward spout to feed. The tip of the spout was 1 cm in front of the platform and 1 cm above it. This arrangement eliminated obstructions between the animal's ears and the loudspeaker while it was eating from the spout. The platform was covered with a piece of damp carpet to provide good traction and facilitate electrical contact with the spout. A contact circuit, connected between the spout and platform, was used to detect when an animal contacted the spout and to activate the syringe pump. Requiring the bat to maintain mouth contact with the spout served to keep its head in a fixed position relative to the loudspeakers.

A shock generator (Coulbourn AC-Resistive Small Animal Shocker) was connected between the reward spout and platform. The shock was adjusted for each individual to the lowest level that consistently caused the bat to back away slightly or lift its head from the spout. Shock levels ranged from 0.05 mA to 0.15 mA. A 25-W light, mounted 0.5 m below the cage, was turned on and off with the shock to provide feedback for a successful avoidance and to indicate when it was safe to return to the spout.

C. Acoustical apparatus

Passive minimum audible angle was determined using a 100-ms broadband noise burst. The noise bursts were digitally generated (Zonic A & D 3525) to include energy up to 100 kHz, and presented through loudspeakers mounted at ear level on a perimeter bar (102 cm radius) centered on the position of an animal's head while it was feeding from the spout. The signal was gated on with an abrupt onset (0.1 ms rise/fall, Coulbourn S84-04), split into left and right channels, amplified to 60 dB sound pressure level (SPL) re 20 µPa (Coulbourn S82-24), and routed to the loudspeakers. During testing, the signal intensity was randomly attenuated up to 3.5 dB on each presentation (Coulbourn S85-08 programmable attenuator) to reduce the possibility of the animals responding on the basis of small intensity differences. The electrical signal going to the speakers was continuously monitored during test sessions with an oscilloscope (Tektronix TDS 210).

Four pairs of ribbon tweeters (Panasonic EAS-10TH100A) that had been matched for similarity of detail in their noise spectra were used. Thus, within a single session, the bats could be tested at four different angles of separation before the loudspeakers had to be moved. The loudspeakers within each matched pair were switched before each session to reduce the possibility that the bats might respond based on speaker quality. At least one pair of loudspeakers was always placed at an angular separation that was too small to be discriminated by the bats to quickly reveal any artifacts that might arise. Failure to discriminate small angles demonstrated that the bats were responding based on locus cues and not quality, intensity, or some unknown artifact.

The ability to use binaural locus cues for localization was assessed using pure tones. Low-frequency tones bend around the head with little or no attenuation, but can be localized by comparing the time of arrival of the phase of each cycle at the two ears (hence the term, phase-difference cue). This cue becomes ambiguous at frequencies for which one half or more cycles of a tone occurs during travel from one ear to the other. The frequency of ambiguity can be calculated using the formula $F = 1/[6(a/C)\sin h]$, where a is the radius of the head (in mm), C is the speed of sound (0.3434 mm/µs), and h is the angle (in radians) of the sound source from the animal's midline (Kuhn, 1977). For common vampire bats (with an approximate maximum interaural delay of 61 µs) all frequencies below 8.5 kHz are calculated to provide an unambiguous phase difference at any angle; at the smaller test angle of 60°, all frequencies below 17 kHz provide an unambiguous cue. These low frequencies are not

attenuated by the head and pinnae, therefore the binaural intensity-difference cue is not available and localization must rely on time cues (Mills, 1972; Plack, 2005). Specifically, sound-level measurements available for this species revealed that any intensity difference below 5 kHz is negligible at an angular separation of $\pm 30^\circ$ (Obrist *et al.*, 1993). Instead, binaural intensity cues are most effective at frequencies whose wavelength is shorter than the head diameter (or presumably pinna dimensions for species with relatively large pinnae). Small intensity differences may be available at somewhat lower frequencies depending on the actual shape of the head and pinnae (for computations, see, e.g., Christensen-Dalsgaard, 2005). For common vampire bats, the intensity-difference cue is calculated to be present at frequencies above approximately 25.7 kHz. Because this is above the frequency of phase ambiguity, it permits assessment of the ability to localize using a pure interaural intensity-difference cue. These values serve as a guide to our interpretation of the performance of the bats with pure-tone stimuli.

The pure tones for this test were generated using a digital tone generator (Zonic A & D 3525). As with the noise, the tones were randomly attenuated over a 3.5-dB range in [1/2]-dB steps (Coulbourn S85-08 programmable attenuator). Because tone localization is difficult for most species, two 100-ms tone pulses were presented on each trial, with the second tone pulse coming 1 s after the first pulse. Each pulse was shaped by a rise-fall gate (Coulbourn S84-04; 10 ms rise/fall) and bandpass filtered (Krohn-Hite 3550; [1/3] octave above and below the frequency of the tone). Finally, the signal was split into left and right channels, separately amplified (Crown D75) and sent to one of a pair of loudspeakers. The acoustic signal at the location of a listening bat was analyzed for overtones using a spectrum analyzer (Zonic A & D 3525); any harmonics in a signal were at least 40 dB below the fundamental frequency and below the animal's detection threshold. Tones were calibrated at the beginning and the end of each test session.

Testing was conducted with the loudspeakers placed 30° to the left and right of midline at the following frequencies: 2, 4, 5, 6.3, 8, 12.5, 16, 20, 40, and 71 kHz. A pair of 6-in. woofers (Infinity RS 2000) was used to produce the 2-kHz tone; higher frequencies were presented using ribbon tweeters (Panasonic EAS10TH400C). The tone pulses were presented at 50 dB above the mean absolute threshold at that frequency except for 2 kHz, which was tested at an intensity of 40 dB above threshold to avoid overtones (Heffner *et al.*, 2013).

Additional tests were conducted with an 8-kHz carrier tone that was sinusoidally amplitude modulated (Krohn-Hite 2400 AM/FM Phase Lock Generator) at 250 Hz and at 500 Hz (100% modulation depth) to provide an ongoing binaural time-difference cue in the envelope of the signal.

D. Sound level measurement

The sound pressure levels of the noise bursts and pure tones (SPL re 20 μPa) were measured before and after the daily testing for each speaker pair using a [1/4]-in. (0.64 cm)

microphone (Brüel & Kjaer 4939), preamplifier (Brüel & Kjaer 2669), measuring amplifier (Brüel & Kjaer 2608), and spectrum analyzer (Zonic A & D 3525). The measuring system was calibrated with a pistonphone (Brüel & Kjaer 4230). Sound measurements were taken by placing the microphone (corrected for protection grid) in the position occupied by the animal's head and pointing it directly toward a loudspeaker (0° incidence). The noise spectrum was relatively flat (± 4 dB) between 3 and 45 kHz, with energy above background level up to 100 kHz (for an illustration of the noise spectra, see Heffner *et al.*, 2007). Thus the signal contained audible energy throughout the hearing range of this species (Heffner *et al.*, 2013).

E. Behavioral procedure

All bats had previously been trained for sound detection and their audiograms determined (Heffner *et al.*, 2013). Thus they were accustomed to the apparatus and to the avoidance task. For sound localization, they were trained to feed from the spout as long as sounds emanated from the right of midline (safe trials). Whenever a sound came from the left of midline (warning trials), they had to break contact to avoid the mild shock, delivered from the spout 2.2 s after signal onset.

Test sessions consisted of a series of 2.2-s trials separated by 0.8-s intertrial intervals. Thus the bats received one noise burst every 3 s. The response of an animal on each trial (i.e., whether or not it made a detection response) was defined as the duration of contact with the spout during the last 150 ms of each 2.2-s trial. If the bat broke contact for more than half of the 150-ms period, a response was recorded. The response was classified as a hit if the preceding signal had come from the animal's left and as a false alarm if it had come from the animal's right. If the bat was not in contact with the spout during the 0.5 s preceding a trial (e.g., if it were grooming), the data from that trial were not recorded even though the trial was presented as usual.

Each trial had a 22% probability of containing a left signal warning of shock. The sequence of left-right trials was quasi-random and is described in detail elsewhere (Heffner and Heffner, 1995; Heffner *et al.*, 2006). Hit and false-alarm rates were determined for each block of approximately 7–8 left trials and 25–35 associated right trials given at a particular 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 \times hit rate). This measure, which can range from 0 (no hits) to 1 (100% hit rate with no false alarms), gives a corrected hit rate by subtracting the proportion of hits that can be attributed to false alarms for each block of trials at each stimulus condition, rather than by the average false alarm rate for a session. This results in a more precise corrected performance measure as false alarm rates normally vary within a session, depending on the animal's satiation and the difficulty of the discrimination.

Noise localization thresholds (minimum audible angle) were determined by gradually reducing the angular separation between the speakers in blocks of trials until a bat could

no longer discriminate reliably between left and right sounds (i.e., the hit rate no longer differed significantly from the false alarm rate, binomial distribution, $p > 0.05$). This was always followed by testing at a larger angle to verify the bat's motivation and continued good performance before again decreasing the angle of separation. Daily testing continued until performance no longer improved at any angle. Asymptotic performance was calculated by averaging the three blocks of trials with the highest scores; these were taken from at least two, and usually three, different sessions. These means were then plotted as the asymptotic performance curve for each bat. Threshold was defined as the angle yielding a performance score of 0.50, which was determined by interpolation. The angles tested were 180°, 120°, 90°, 60°, 30°, 20°, 15°, 10°, and 5°.

Localization performance for pure-tones and amplitude modulated tones was determined using a fixed angle of 60° separation, a relatively easy angle for this species and one that allows direct comparison with data from many other species (e.g., Masterton *et al.*, 1975). Pure tones throughout the hearing range were used as long as they could be produced at 40–50 dB above threshold without distortion. Testing was carried out in blocks of eight warning trials with a single frequency per session for frequencies that sustained good performance. However, if a bat had difficulty or was unable to localize a particular frequency, broadband noise was presented for several trials intermittently throughout the session to verify that the bat remained sufficiently motivated to respond to localizable sounds. Each frequency was tested for at least four sessions for 120–216 warning trials for bats A and B; bat C was a reluctant performer in the tone-localization task and data were obtained in two to four sessions. The top 50% of the trial blocks for each frequency was then averaged to represent the best overall performance each bat was capable of sustaining.

III. RESULTS

A. Minimum audible angle

Figure 1 illustrates the performance for the three common vampire bats localizing a single 100-ms noise burst. The three individuals showed excellent agreement consistent with our experience that healthy, well-trained observers of the same species do not vary much in their auditory abilities. All three bats were capable of excellent performance at angles of 90° and greater separation, thereby demonstrating good motivation and ability to perform the behavioral task. Performance declined rapidly at angles smaller than 30° and the mean threshold for the three bats was 13.1°.

B. Use of binaural locus cues

Figure 2 shows the performances of the vampire bats localizing pure tones as well as the 8-kHz tone at two rates of amplitude modulation. As indicated by the arrows just above the x axis, at an angular separation of 60°, the binaural phase-difference was physically unambiguous up to 17 kHz. Binaural intensity differences were calculated to be available at frequencies above approximately 25.7 kHz. These values

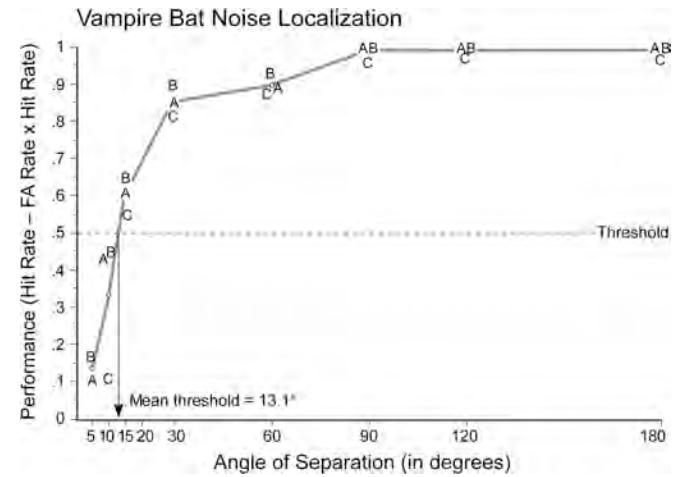


FIG. 1. Sound-localization performance of three common vampire bats (*Desmodus rotundus*). The performances of the three individuals are plotted with the line representing the mean. The 50% corrected detection threshold is 13.1°. Chance performance for this task was approximately 30%–35% corrected detection.

serve as a guide to our interpretation of the performance of the bats with pure-tone stimuli.

Again the three bats showed good agreement in their performances and easily localized low and high frequencies as well as the amplitude modulated tones. Their performance at low frequencies (2 and 4 kHz) was similar to their performance with the broadband noise at an angle of 60° separation, indicating that this species, unlike many other bat species, is able to use the binaural phase-difference cue. As frequency increased through the midrange of hearing, performance declined. The highest frequency that could be consistently localized using the phase-difference cue was 5 kHz,

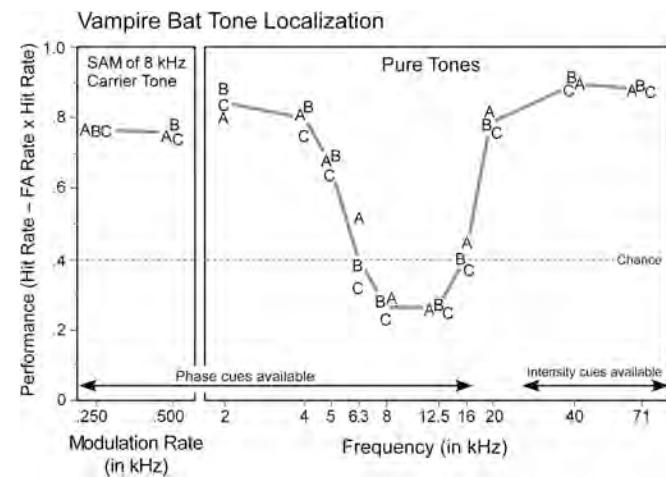


FIG. 2. Sound-localization performance at 60° separation for three common vampire bats as a function of the frequency of a pure tone stimulus or the frequency of sinusoidal amplitude modulation (SAM) of an 8-kHz tone. The ability of the bats to localize high- and low-frequency pure tones demonstrates their ability to use both the binaural intensity and time locus cues, respectively. Although they were unable to use either of the binaural cues to localize pure tones from 6.3 to 16 kHz, they were able to localize 8 kHz if a time cue was introduced by modulating the tone at 250 or 500 Hz. Note that vampire bats can barely detect a 500-Hz pure tone at 70 dB SPL and are unable to hear 250-Hz tones (Heffner *et al.*, 2013). Arrows indicating cue availability are estimates based on physical calculations.

and performance fell to chance from 6.3 to 16 kHz, even though the cue was physically available at these frequencies. As pure-tone frequency increased further and the binaural intensity cue became available, performance rose and again reached 90% or higher corrected detection. The ability to localize pure tones above the frequency of phase ambiguity showed good use of the binaural intensity-difference cue. As a further test of their ability to use the phase cue, an 8-kHz pure tone, which they could not localize, was amplitude modulated at 250 and 500 Hz. With the low-frequency-modulated envelope, performance improved dramatically, showing that they can extract a binaural time difference using the envelope of a sound regardless of the localizability of the carrier tone.

IV. DISCUSSION

A. Acuity

The mean minimum audible angle for passive sound localization for the three common vampire bats is 13.1°. This threshold places them within the range of 9.5° to 14.7° observed in seven other species of bats, including both echolocators and non-echolocators (Table I). This threshold also

TABLE I. Functional head size (the time it takes for sound to travel around the head from one ear to the other), sound localization acuity, and the highest frequency at which the binaural phase-difference cue is used in bats.

Species	Functional head size (μs)	Minimum audible angle (deg.)	Highest frequency using binaural phase cue (kHz) ⁱ
Echolocators			
Greater spear-nosed bat ^{a,b} (<i>Phyllostomus hastatus</i>)	108	9.5	Cue not used
Jamaican fruit bat ^c (<i>Artibeus jamaicensis</i>)	89	9.9	6.3
Common vampire bat ^d (<i>Desmodus rotundus</i>)	61	13.1	5.0
Big brown bat ^e (<i>Eptesicus fuscus</i>)	50	14	Cue not used
Short-tailed fruit bat ^{a,b} (<i>Carollia perspicillata</i>)	47	14.7	Cue not used
Atypical echolocator			
Egyptian fruit bat ^f (<i>Rousettus aegyptiacus</i>)	135	11.6	5.6
Non-echolocators			
Straw-colored fruit bat ^g (<i>Eidolon helvum</i>)	145	11.7	Cue not used
Dog-faced fruit bat ^{g,h} (<i>Cynopterus brachyotis</i>)	86	10.5	Cue not used

^aHeffner et al., 2007.

^bHeffner et al., 2010b.

^cHeffner et al., 2001c.

^dCurrent report.

^eKoay et al., 1998.

^fHeffner et al., 1999.

^gHeffner et al., 2008.

^hHeffner et al., 2010a.

ⁱThis value is the highest frequency that could be localized using the binaural phase cue; the next higher test frequency (within one octave or less) was not localized above chance. Thus, the true limit could be slightly higher depending on the actual frequencies tested.

places vampire bats at the median for more than 40 terrestrial mammals. The relatively good passive localization acuity of such small species as vampire bats and other small bats supports the observation that the size of the available binaural cues is not the determining factor in sound-localization acuity (Heffner and Heffner, 1992a). Sound-localization acuity near the mean for mammals is possible even in mammals whose interaural distances are among the smallest. It is likely that pinna cues contribute to their good acuity, especially when the signal includes frequencies of 25–35 kHz, for which the pinna of this species is highly directional (Obrist et al., 1993).

It is important to note that the variation in sound localization acuity is not accounted for by differences in the magnitude of the sound localization cues that result from differences in head size, but to selective pressures involved in the evolution of hearing. Specifically, the variation in sound localization is closely correlated with the width of an animal's visual field such that animals with narrow fields of best vision have better sound localization acuity than those with wider fields of best vision (Heffner and Heffner, 1992c; Heffner and Heffner, 2003; Heffner et al., 2008). This holds true regardless of the magnitude of the binaural cues for locus available to an animal and regardless of whether a species uses only one or both of the binaural cues for localization. It also applies to echolocating and non-echolocating bats, even though echolocators do not rely on vision as much as most mammals and their best visual acuity is relatively poor (Heffner et al., 2007; Pettigrew et al., 1988). Although we do not have direct data on the distribution of resolution across the retina of the common vampire bat, their vision is thought to be very similar to that of other Phyllostomidae (Bell and Fenton, 1986; Hope and Bhatnagar, 1979; Manske and Schmidt, 1976; Schmidt, 1988) and is likely to be consistent with the reported relationship between localization acuity and field of best vision (Heffner et al., 2008).

It may be noted that the 13.1° threshold reported here for the common vampire bat, as well as the minimum audible angles for other bats (Table I), are larger than the 2° localization accuracy reported by Fuzessery and his colleagues for the pallid bat, *Antrozous pallidus* (Fuzessery et al., 1993; Barber et al., 2003). The reason for this lies in the different nature of the measurements themselves. Specifically, the pallid bats were tested by having them fly to the source of a brief sound, which gives a measure of accuracy. The bats in our studies were tested by determining the smallest angular separation they could resolve (that is, the minimum audible angle), which gives a measure of precision or acuity. Minimum audible angle has long been used in psychophysics and has proven to be a useful measure of sound localization not only for comparing different species, but also for studying the impact of auditory neural damage on sound localization and hearing (e.g., Heffner and Heffner, 2014; Heffner et al., 2001b; Koay et al., 2002b). In addition, it is often easier to rule out potential confounds when determining minimum audible angle, especially when using conditioned suppression/avoidance. For example, in training their bats to fly to the source of a sound to obtain a mealworm, Barber et al. (2003) reduced or eliminated the use of

vision by conducting their experiments in red light and used dummy mealworms made of duct tape. The use of olfaction was controlled by coating the dummy mealworms with mealworm paste. Detectable echolocation calls were recorded, which showed that although the bats emitted echolocation calls during the first half of a trial, no calls were detected on their final approach to the food. Such potential use of other senses is inherently ruled out in the conditioned suppression/avoidance minimum audible angle task. That is, there are no potential visual or olfactory cues and the bats in the present study could not echolocate while they were eating at the food spout. Moreover, the determination of minimum audible angle includes trials at angles below threshold, a procedure that unequivocally demonstrates that animals are discriminating the locus of a sound source and not artifacts. Finally, the conditioned suppression/avoidance procedure makes minimal demands on an animal's cognitive and motor skills, as it uses the natural freezing response that virtually all animals show when they detect danger. Thus, determining the minimum audible angle using conditioned suppression/avoidance provides a uniform procedure for comparing sound localization acuity of most species of mammal or bird.

B. Use of binaural locus cues

Common vampire bats are able to localize both high- and low-frequency pure tones at 60° separation, showing excellent performance that is comparable to their performance with broadband noise at that angle. This result indicates they can use not only the high-frequency-dependent binaural intensity-difference cue, but that they can also use the low-frequency binaural phase-difference cue. Common vampire bats use the phase-difference cue at frequencies from 2 kHz (the lowest frequency tested) to 5 kHz. The inability of the bats to localize intermediate frequencies from 6.3–16 kHz indicates that they could use neither binaural cue at those frequencies. When frequency was increased further to 20 kHz, at which the binaural intensity cue is estimated to be physically available to provide a basis for localization (e.g., Christensen-Dalsgaard, 2005), performance again became excellent.

It may be noted that there is another binaural locus cue, the onset cue, which occurs between the time a sound reaches the near ear and the later arrival of the sound at the far ear. Although the onset cue is sometimes thought of as a binaural time-difference cue, it appears to be a transient intensity-difference cue (Elfner and Tomsic, 1968; Perrott, 1969). Moreover, it is a weak cue that at best allows an animal to maintain performance above chance levels, but well below its asymptotic abilities (Wesolek *et al.*, 2010). Because the bats' performances fell to chance at 6.3 to 16 kHz, they could not have been using an onset cue for those midrange frequencies. Thus, there is little reason to attribute their good performance at frequencies below or above that range to use of a transient onset difference.

Nor can the bats' ability to localize frequencies below 6.3 kHz be attributed to any small ongoing interaural intensity differences that might exist at low frequencies. Free-

field tone localization tests have long been used to investigate the ability of humans and animals to use the binaural locus cues (e.g., Masterton *et al.*, 1975) and the available binaural intensity difference declines steadily as frequency is decreased for large and small animals (e.g., Mills, 1958; Obrist *et al.*, 1993; Wesolek *et al.*, 2010). For example, interaural intensity differences have been measured in a variety of bat species at frequencies as low as 5 kHz, with differences at lower frequencies being unmeasurable. Those measures revealed differences of 3 dB or less at angular separations of 90° and requiring, in some cases, a 76° separation to achieve a difference of only 1 dB (Obrist *et al.*, 1993). Thus, one would not expect to see the binaural intensity cue become unusable at 16 kHz and then reappear at 2 and 4 kHz, as shown in Fig. 2.

Finally, although we noted above that the binaural phase cue is a "low-frequency" locus cue, this does not mean that the binaural time cue requires low-frequency sounds. As shown in Fig. 2, the bats were easily able to localize 8 kHz when the signal was modulated at 250 or 500 Hz. This finding is consistent with the human studies showing that high frequency noise and tone complexes can be lateralized on the basis of interaural time differences (e.g., McFadden and Passanen, 1976). That is, while the binaural time cue is available from low-frequency pure tones up to the ability of the nervous system to perform a cycle-by-cycle analysis of the binaural input, it is also available from any complex signal, high or low frequency, in which the envelope of the signal varies sufficiently such that the nervous system can match the waveform arriving at one ear with the arrival of the same part of the waveform at the other ear. Because the analysis of the binaural time cue available in low-frequency pure tones is performed on a cycle-by-cycle basis, it is often referred to as the binaural phase cue. However, there is no reason to believe that the nervous system determines the binaural arrival times of sine waves differently from those of other sounds. As such, we view the ability to localize low-frequency pure tones as a test of the ability to use the binaural time cue in general.

1. Does interaural distance determine the use of time cues?

It may be noted that those species that show no evidence of using the binaural time cues are all small with comparatively small heads, suggesting that perhaps their functional interaural distances may be too small for the nervous system to extract useful time information. The common vampire bat is now the smallest mammalian species so far to use time cues (Fig. 3). It is also the third bat to demonstrate an ability to localize using time cues, with five other species of bat demonstrating that they cannot use the cue (Table I). Thus, small head size and the accompanying small interaural delays do not appear to impose an insurmountable constraint on the ability to use the delays for localization. Indeed, the delay available to the common vampire bat at the test angle of $\pm 30^\circ$ is approximately $20\ \mu s$, which is near the threshold delay for humans using either broad band noise or pure tones (Klumpp and Eady, 1956), and well below the $50\text{--}60\ \mu s$

interaural time-difference thresholds of domestic rabbits obtained using earphones (Ebert *et al.*, 2008). Figure 3 reveals that the maximum functional interaural distances of species capable of using time cues (shown on the left) fall well within the range of interaural distances of species that do not use time cues (shown on the right). Many species retain the ability to process interaural time delays for localization despite having only very short delays available to them. This includes not only the common vampire bat with a calculated maximum interaural delay of about $61\ \mu\text{s}$, but also Jamaican fruit bats, *Artibeus jamaicensis* ($89\ \mu\text{s}$), gerbils ($87\ \mu\text{s}$), and least weasels ($76\ \mu\text{s}$) (Heffner and Heffner, 1987, 1988; Heffner *et al.*, 2001c). Thus, although the absence of the binaural time cue has been found only in very small mammals, a small interaural distance, with its consequent small interaural time delays, does not seem an insurmountable obstacle to using time cues for sound localization, just as it is not an obstacle to good localization acuity. Other factors must be involved.

Neural responses to time delays have been recorded in many species and at many levels of the auditory system, with the goal of finding the mechanism(s) underlying the ability to use these delays for localization. These studies are usually limited by a lack of data on the behavioral abilities of the recorded species so that we cannot always relate the neural responses to behavioral functions. In many cases, neural responses are elicited to delays much longer than can occur naturally, further complicating their interpretation and leading some to suggest the responses may be related to functions other than sound localization, or may even be epiphenomena (e.g., Grothe and Neuweiler, 2000). Useful insight into the significance of the neural responses to interaural delays could be gained by comparing responses in closely related species that nevertheless differ in their ability to use binaural time cues. For example, within the family Phyllostomidae, common vampire bats and Jamaican fruit bats both use the binaural time cue, whereas short-tailed fruit bats (*Carollia perspicillata*) and greater spear-nosed bats (*Phyllostomus hastatus*) do not use the cue. Among the Pteropodidae, the Egyptian fruit bat (*Rousettus aegyptiacus*) uses the binaural time cue whereas the straw-colored fruit bat (*Eidolon helvum*) and dog-faced fruit bat (*Cynopterus brachyotis*) do not (Fig. 3). Such comparative studies, coupled with recognition of species differences in localization acuity, would circumvent the temptation to use the neural responses of one species with poor acuity, such as rabbits, to explain the good acuity of another, such as humans (Skottun, 1998).

2. The medial superior olfactory nucleus and the use of time cues

The neural analysis of binaural locus cues relies on a convergence of the input from the two ears that first occurs in the superior olfactory complex (e.g., Erulkar, 1972; Irvine, 1992). Of the nuclei in this complex, the medial superior olfactory nucleus (MSO) is the site at which the temporal relation between the inputs is encoded. Based on this view, Masterton and his colleagues assessed the ability of four species of mammals to use the binaural time-difference cue.

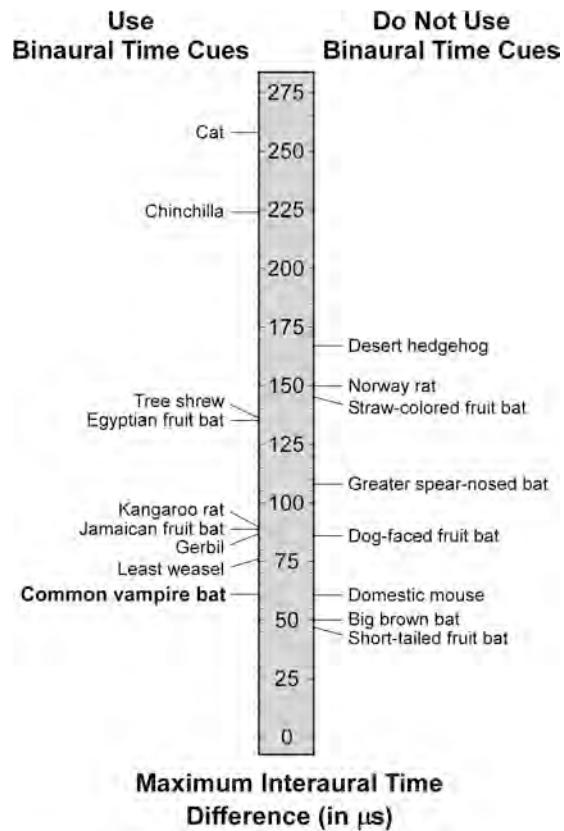


FIG. 3. Use of the binaural phase-difference cue among small to medium sized mammals; all larger species tested so far have been able to use this cue. The common vampire bat, with its estimated interaural delay of $61\ \mu\text{s}$, is the smallest species to use time cues in the form of interaural phase-differences. Note that there is considerable overlap in the calculated maximum interaural delays of species that use the cue versus species that do not use the cue.

They did this by determining the ability to localize pure tones that were too low in frequency to be localized using the intensity-difference cue, thereby requiring the animals to rely on a time cue in the form of a phase difference (Masterton *et al.*, 1975). The asymptotic performances of the four species varied directly with the relative size of their MSO. Cats had the best performance and largest MSO, followed in order by tree shrews and rats; hedgehogs, which lack an MSO, were completely unable to localize low-frequency tones. Although this suggested that the primary, if not sole, purpose of the MSO is to analyze binaural time differences, that interpretation was weakened by more recent testing (with better control of acoustic artifacts) that revealed rats to be unable to use the phase cue despite the presence of a small MSO (Wesolek *et al.*, 2010). Other exceptions to a simple correspondence between the presence of an MSO and the use of the binaural phase cue have also begun to appear, making it worthwhile to reexamine the relation between the presence of an MSO and the use of binaural time cues in the light of new data.

The MSO is an extraordinarily variable nucleus and the question of whether an animal possesses an MSO depends on how it is identified (Grothe and Park, 2000). The classic anatomical description of the MSO is that of a distinct sheet of bipolar (or disk shaped) neurons located medially to the

lateral superior olivary nucleus, with dendrites extending medially and laterally, receiving bilateral input from the anteroventral cochlear nuclei, and sending output to the ipsilateral inferior colliculus (e.g., Grothe, 2000; Schwartz, 1992). The important physiological characteristics of an MSO are sensitivity to interaural time differences, responses limited primarily to low frequencies, excitatory responses to input from each ear, and a more recent recognition of the importance of bilateral inhibition (Brand *et al.*, 2002; Grothe, 2003). However, there appear to be many likely exceptions to this typical configuration. For example, we know that some MSO cells in some species respond to higher frequencies and in “atypical” fashion, and the MSOs of some small mammals are “non-classic” in that they do not possess all these standard features, leading some to suggest that those MSOs are not involved in the analysis of interaural time differences for sound localization (e.g., Grothe, 2000; Grothe and Park, 2000; Grothe *et al.*, 2010). Although the auditory brainstems of many species have been studied (e.g., Baron *et al.*, 1996; Grothe, 2000; Schwartz, 1992), not all of the anatomical and physiological characteristics are known for each species. For example, the presence of an MSO is often based on the location and appearance of a cell group, with little information on its anatomical connections or the binaural response properties of its neurons. Nevertheless, based on the limited information now available for bats, we can search for correspondence between the presence of an MSO and the ability to use binaural time cues by determining whether those animals that are known to use the time cue are thought to have an MSO and those that do not use the cue appear to lack an MSO. Considering the eight species in Fig. 3 that use binaural time cues, all but one are known to have an MSO, including the vampire bat (Kuwabara and Bhatnagar, 1999). The only exception, the Jamaican fruit bat, does not have an identifiable MSO (Zook and Casseday, 1980), yet it is able to localize low-frequency pure tones for which an interaural phase difference is the only available cue (Heffner *et al.*, 2001c). The neural structures in this species that analyze the interaural time differences are not known. It is possible that more powerful anatomical techniques would reveal a nucleus that might be classified as an MSO. Alternatively, cells in the LSO that respond to time differences have been reported (e.g., Joris and Yin, 1995) and may have become more prominent in this species and more sensitive to very small delays, thereby enabling it to use time cues in the absence of an MSO.

Of the eight species in Fig. 3 that do not use binaural time cues, information about the MSO is available for only five. Of these, only the hedgehog lacks an MSO, whereas the other four species, the laboratory rat, laboratory mouse, short-tailed fruit bat, and big brown bat (*Eptesicus fuscus*) are reported to have an MSO (e.g., Grothe *et al.*, 2001; Huffman and Covey, 1995; Hutson, 2000; Masterton *et al.*, 1975). However, the MSO’s in each of these species departs from the classic MSO. For example, the MSO of the rat does not have the expected cytoarchitecture, much of its input is monaural, and its inhibitory input is atypical (Grothe, 2003; Inbody and Feng, 1981; Kapfer *et al.*, 2002). The MSO of the mouse is the smallest relative to its brain size among 53

mammals (Glendenning and Masterton, 1998). The big brown bat’s MSO is also very small, with only about half of the cells receiving binaural input, and its output is bilateral rather than ipsilateral (Covey, 2005; Grothe *et al.*, 2001; Huffman and Covey, 1995)—all of which are atypical features that call into question its participation in a binaural time analysis for localization. Finally, the MSO of the short-tailed fruit bat, although described as “prominent,” is based on its microscopic appearance; its physiology and anatomical connections are unknown (Hutson, 2000), leaving open the question of whether it is homologous to that of species known to use time cues. Whether or not the MSO’s of these four species function in the classic manner, we leave for others to determine. Yet we now know that users as well as non-users of time cues include species that have an MSO and species that do not. Taken together we can only conclude that our understanding of the role of the MSO in processing binaural cues for sound localization remains incomplete and the MSO may have functions in addition to binaural time analysis (e.g., Grothe, 2000). With the possibility for multiple functions within the MSO, it seems likely that different functions may be retained and lost in different species, thereby accounting for the different appearance of the nucleus and the variation in ability among species that possess what at first appears to be the “same” nucleus. Knowing this, we can take advantage of the variation in cytoarchitecture, innervation, and ability, especially among bats (Baron *et al.*, 1996), to better understand both the mechanisms underlying sound localization and its evolution.

3. Low-frequency hearing and use of the binaural phase-difference cue

The highest-frequency pure tone that an animal can localize using the binaural phase cue is of interest because it indicates that its auditory system is able to follow, or phase lock, at a frequency at least that high. Not only does the upper limit of the binaural phase cue vary between species, but it appears to be related to an animal’s ability to hear low-frequency sounds. To understand this relationship, it must be noted that low-frequency hearing limits, defined as the lowest frequency audible at 60 dB SPL, are bimodally distributed—one group with good low-frequency hearing is able to hear below 125 Hz and another group with poor low-frequency hearing is typically unable to hear much below 500 Hz (Heffner *et al.*, 2001a, 2013). Although little is known about the significance of this dichotomy in low-frequency hearing, we have suggested it may demarcate those animals that do, and those that do not use temporal processing for encoding pitch. Taking this dichotomy into account, it can be seen that all species tested so far that have good low-frequency hearing also use the binaural phase cue. On the other hand, whereas the majority of species that do not have good low-frequency hearing do not use the binaural phase cue, there are some exceptions. As shown in Fig. 4, there are three species of bats, including the common vampire, with 60-dB SPL hearing limits extending only as low as 2800 to 710 Hz, that nevertheless do use the binaural phase cue. Clearly, the absence of good low-frequency hearing

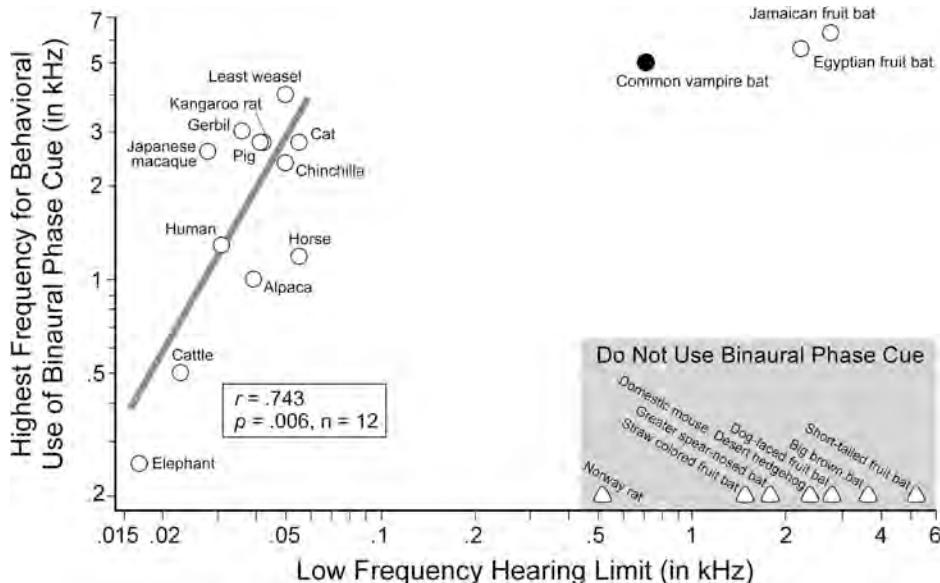


FIG. 4. Relation between lowest audible frequency at 60 dB SPL and the highest frequency at which a species can use the binaural phase-difference cue. All species that hear below 125 Hz are able to use the phase cue and most species that do not hear below about 500 Hz are unable to use the phase cue. However, at least three species with poor low-frequency hearing are able to use the cue. Thus, low-frequency hearing is not a good predictor of the ability or inability to use the phase cue. However, it does predict the highest frequency at which the cue can be used among those species whose low-frequency hearing extends below 0.125 kHz.

does not preclude the use of the binaural phase-difference cue and this raises questions about the limits of neural phase locking on which the phase cue is thought to depend.

4. Upper limit of phase locking in the auditory system

If an animal is to use the binaural phase cue, then it must hear frequencies low enough to permit synchronous firing (neural phase locking) to support a binaural phase comparison. Similarly, the highest frequency at which the cue can be used would seem to be affected by the ability of the nervous system to phase lock at high frequencies. In other words, a species should be able to use the phase cue at frequencies as high as the highest frequency at which its auditory system can phase lock, as long as the cue is physically unambiguous. It has been shown that the upper limit of strong phase locking in mammals is about 3 kHz, with synchrony statistically detectable up to about 5 kHz (e.g., Bremen and Joris, 2013; Grothe *et al.*, 2010; Simmons and Simmons, 2011). If we accept that the highest frequency at which the phase cue is used is an estimate of the upper limit of phase locking, then bats reveal that useful phase locking can occur at frequencies up to 6.3 kHz in the Jamaican fruit bat, which is somewhat higher than heretofore seen in mammals (Fig. 4). Since so few bats have been examined for their ability to use the binaural phase-difference cue, higher frequencies may yet be demonstrated. This suggests that a comparative exploration of physiological phase locking in a wider range of species might reveal a capacity for phase locking in mammals at higher frequencies than currently recognized and lead us to new questions regarding the selective pressure to develop such abilities. Yet we must also recognize the substantial number of species with similar auditory sensitivities that forego the use of the phase cue and presumably phase locking (gray region in Fig. 4); understanding the difference between those species and the three bats in the upper right quadrant of Fig. 4 may provide unexpected insight into basic hearing capabilities of mammals.

This brings us, finally, to ask, what does limit the use of the binaural phase cue in mammals? For small mammals that use the binaural phase-difference cue, there is always a range of unlocalizable frequencies for which a phase difference is nevertheless physically present and unambiguous (less than one-half cycle occurs during the interaural delay). For common vampire bats these frequencies are between approximately 6.3 and 17 kHz, based on a spherical head model and sound sources 30° from the midline. This gap in localizable pure tone frequencies is not unusual among bats and other small species that use the phase-difference cue, including chinchillas, Jamaican and Egyptian fruit bats, and least weasels (Heffner and Heffner, 1987; Heffner *et al.*, 1994, 1999, 2001c). It seems that none of the smaller species examined so far use the phase cue throughout the range for which it is physically available. Thus it follows that even if a phase difference is physically present and unambiguous, the nervous system may not make use of it throughout the entire range over which it is available. It is likely that the physiological limits may extend to somewhat higher frequencies than so far recorded, as indicated by the bats that use the cue above 5 kHz. However, phase locking may not extend far beyond this point because there may be little selective pressure to do so, since sounds that are above the range of an animal's ability to use the binaural phase cue can still be localized using a binaural time cue if they are modulated, as is the case for most natural sounds, including both transients and communication calls.

ACKNOWLEDGMENTS

Supported by NIH R15-DC009321. We thank the Milwaukee County Zoo for the use of these bats for behavioral research. We also thank Steve Wing for his helpful manual for the maintenance and husbandry of common vampire bats.

Barber, J. R., Razak, K. A., and Fuzessery, Z. M. (2003). "Can two streams of auditory information be processed simultaneously? Evidence from the gleaning bat *Antrozous pallidus*," *J. Comp. Physiol. A* **189**, 843–855.

- Barnard, S. M. (1995). *Bats in Captivity* (Wild Ones Animal Books, Springville, CA), pp. 77–94.
- Baron, G., Stephan, H., and Frahm, H. D. (Eds.) (1996). *Comparative Neurobiology in Chiroptera* (Birkhäuser, Basel), Vol. 3, pp. 1075–1596.
- Bell, G. P., and Fenton, M. B. (1986). “Visual acuity, sensitivity and binocularity in a gleaning insectivorous bat, *Macrotus californicus* (Chiroptera: Phyllostomidae).” *Anim. Behav.* **34**, 409–414.
- Brand, A., Behrend, O., Marquardt, T., McAlpine, D., and Grothe, B. (2002). “Precise inhibition is essential for microsecond interaural time difference coding.” *Nature* **417**, 543–547.
- Bremen, P., and Joris, P. X. (2013). “Axonal recordings from medial superior olive neurons obtained from the lateral lemniscus of the chinchilla (*Chinchilla laniger*).” *J. Neurosci.* **33**, 17506–17518.
- Christensen-Dalsgaard, J. (2005). “Directional hearing in nonmammalian tetrapods,” in *Sound Source Localization*, edited by A. N. Popper and R. R. Fay (Springer Science, New York), pp. 67–123.
- Covey, E. (2005). “Neurobiological specializations in echolocating bats,” *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **287**, 1103–1116.
- Ebert, C. S., Blanks, D. A., Patel, M. R., Coffey, C. S., Marshall, A. F., and Fitzpatrick, D. C. (2008). “Behavioral sensitivity to interaural time differences in the rabbit.” *Hear. Res.* **235**, 134–142.
- Elfner, L. F., and Tomsic, R. T. (1968). “Temporal and intensive factors in binaural lateralization of auditory transients.” *J. Acoust. Soc. Am.* **43**, 746–751.
- Erulkar, S. D. (1972). “Comparative aspects of spatial localization of sound.” *Physiol. Rev.* **52**, 236–360.
- Fuzessery, Z. M., Buttenhoff, P., Andrews, B., and Kennedy, J. M. (1993). “Passive sound localization of prey by the Pallid bat (*Antrozous p. pallidus*).” *J. Comp. Physiol. A* **171**, 767–777.
- Glendenning, K. K., and Masterton, R. B. (1998). “Comparative morphometry of mammalian central auditory systems: Variation in nuclei and form of the ascending system.” *Brain Behav. Evol.* **51**, 59–89.
- Grothe, B. (2000). “The evolution of temporal processing in the medial superior olive, an auditory brainstem structure.” *Prog. Neurobiol.* **61**, 581–610.
- Grothe, B. (2003). “New roles for synaptic inhibition in sound localization.” *Nat. Rev. Neurosci.* **4**, 540–550.
- Grothe, B., Covey, E., and Casseday, H. H. (2001). “Medial superior olive of the Big brown bat: Neuronal responses to pure tones, amplitude modulations, and pulse trains.” *J. Neurophysiol.* **86**, 2219–2230.
- Grothe, B., and Neuweiler, G. (2000). “The function of the medial superior olive in small mammals: Temporal receptive fields in auditory analysis.” *J. Comp. Physiol. A* **186**, 413–423.
- Grothe, B., and Park, T. J. (2000). “Structure and function of the bat superior olfactory complex.” *Microsc. Res. Tech.* **51**, 382–402.
- Grothe, B., Pecka, M., and McAlpine, D. (2010). “Mechanisms of sound localization in mammals.” *Physiol. Rev.* **90**, 983–1012.
- Heffner, R. E., and Heffner, R. S. (1995). “Conditioned avoidance,” in *Methods in Comparative Psychoacoustics*, edited by G. M. Klump, R. J. Dooling, R. R. Fay, and W. C. Stebbins (Birkhäuser, Basel), pp. 79–93.
- Heffner, R. E., and Heffner, R. S. (2003). “Audition,” in *Handbook of Research Methods in Experimental Psychology*, edited by S. Davis (Blackwell, Boston, MA), pp. 413–440.
- Heffner, R. E., and Heffner, R. S. (2014). “The behavioral study of mammalian hearing,” in *Perspectives on Auditory Research*, edited by A. N. Popper and R. R. Fay (Springer, New York), pp. 269–285.
- Heffner, R. S., and Heffner, H. E. (1982). “Hearing in the elephant (*Elephas maximus*): Absolute sensitivity, frequency discrimination, and sound localization.” *J. Comp. Physiol. Psychol.* **96**, 926–944.
- Heffner, R. S., and Heffner, H. E. (1986). “Localization of tones by horses: Use of binaural cues and the role of the superior olfactory complex.” *Behav. Neurosci.* **100**, 93–103.
- Heffner, R. S., and Heffner, H. E. (1987). “Localization of noise, use of binaural cues, and a description of the superior olfactory complex in the smallest carnivore, the Least weasel (*Mustela nivalis*).” *Behav. Neurosci.* **101**, 701–708, 744, 745.
- Heffner, R. S., and Heffner, H. E. (1988). “Sound localization and use of binaural cues by the gerbil (*Meriones unguiculatus*).” *Behav. Neurosci.* **102**, 422–428.
- Heffner, R. S., and Heffner, H. E. (1989). “Sound localization, use of binaural cues and the superior olfactory complex in pigs.” *Brain Behav. Evol.* **33**, 248–258.
- Heffner, R. S., and Heffner, H. E. (1990). “Vestigial hearing in a fossorial mammal, the pocket gopher (*Geomys bursarius*).” *Hear. Res.* **46**, 239–252.
- Heffner, R. S., and Heffner, H. E. (1992a). “Evolution of sound localization in mammals,” in *The Evolutionary Biology of Hearing*, edited by D. B. Webster, R. R. Fay, and A. N. Popper (Springer, New York), pp. 691–715.
- Heffner, R. S., and Heffner, H. E. (1992b). “Hearing and sound localization in Blind mole rats, *Spalax ehrenbergi*.” *Hear. Res.* **62**, 206–216.
- Heffner, R. S., and Heffner, H. E. (1992c). “Visual factors in sound localization in mammals.” *J. Comp. Neurol.* **317**, 219–232.
- Heffner, R. S., and Heffner, H. E. (1993). “Degenerate hearing and sound localization in Naked mole rats (*Heterocephalus glaber*), with an overview of central auditory structures.” *J. Comp. Neurol.* **331**, 418–433.
- Heffner, R. S., Heffner, H. E., Kearns, D., Vogel, J., and Koay, G. (1994). “Sound localization in chinchillas, I: Left/right discriminations.” *Hear. Res.* **80**, 247–257.
- Heffner, R. S., Koay, G., and Heffner, H. E. (1999). “Sound localization in an Old-world fruit bat (*Rousettus aegyptiacus*): Acuity, use of binaural cues, and relationship to vision.” *J. Comp. Psychol.* **113**, 297–306.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2001a). “Audiograms of five species of rodents: Implications for the evolution of hearing and the encoding of pitch.” *Hear. Res.* **157**, 138–152.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2001b). “Sound-localization acuity changes with age in C5BL/6J mice,” in *The Auditory Biology of the Laboratory Mouse: From Behavior to Molecular Biology*, edited by J. Willott (CRC Press, New York), pp. 31–35.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2001c). “Sound localization in a New-world frugivorous bat, *Artibeus jamaicensis*: Acuity, use of binaural cues, and relationship to vision,” *J. Acoust. Soc. Am.* **109**, 412–421.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2006). “Hearing in large (*Eidolon helvum*) and small (*Cynopterus brachyotis*) non-echolocating fruit bats.” *Hear. Res.* **221**, 17–25.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2007). “Sound-localization acuity and its relation to vision in large and small fruit-eating bats: I. Echolocating species, *Phyllostomus hastatus* and *Carollia perspicillata*,” *Hear. Res.* **234**, 1–9.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2008). “Sound-localization acuity and its relation to vision in large and small fruit-eating bats: II. Non-echolocating species, *Eidolon helvum* and *Cynopterus brachyotis*,” *Hear. Res.* **241**, 80–86.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2010a). “Use of binaural cues for sound localization in large and small non-echolocating bats: *Eidolon helvum* and *Cynopterus brachyotis*,” *J. Acoust. Soc. Am.* **127**, 3837–3845.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2010b). “Use of binaural cues for sound localization in two species of Phyllostomidae: The Greater spear-nosed bat (*Phyllostomus hastatus*) and the Short-tailed fruit bat (*Carollia perspicillata*).” *J. Comp. Psychol.* **124**, 447–454.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2013). “Hearing in American leaf-nosed bats. IV: The common vampire bat, *Desmodus rotundus*,” *Hear. Res.* **296**, 42–50.
- Heffner, R. S., Koay, G., and Heffner, H. E. (2014). “Hearing in alpacas (*Vicugna pacos*): Audiogram, localization acuity, and use of binaural locus cues.” *J. Acoust. Soc. Am.* **135**, 778–788.
- Hope, G. M., and Bhatnagar, K. P. (1979). “Electrical response of bat retina to spectral stimulation: Comparison of four microchiropteran species.” *Experientia* **35**, 1189–1191.
- Huffman, R. F., and Covey, E. (1995). “Origin of ascending projections to the nuclei of the lateral lemniscus in the Big brown bat, *Eptesicus fuscus*,” *J. Comp. Neurol.* **357**, 532–545.
- Hutson, K. A. (2000). “Auditory brainstem nuclei in a New World fruit bat: *Carollia perspicillata*,” *Neuroscience Abstracts* No. 252.9.
- Inbody, S. B., and Feng, A. S. (1981). “Binaural response characteristics of single neurons in the medial superior olfactory nucleus of the albino rat.” *Brain Res.* **210**, 361–366.
- Irvine, D. R. F. (1992). “Physiology of the auditory brainstem,” in *The Mammalian Auditory Pathway: Neurophysiology*, edited by A. N. Popper and R. R. Fay (Springer, New York), pp. 153–231.
- Joris, P. X., and Yin, T. C. (1995). “Envelope coding in the lateral superior olive: I. Sensitivity to interaural time differences.” *J. Neurophysiol.* **73**, 1043–1062.
- Kapfer, C., Seidl, A. H., Schweizer, H., and Grothe, B. (2002). “Experience-dependent refinement of inhibitory inputs to auditory coincidence-detector neurons.” *Nat. Neurosci.* **5**, 247–253.
- Klumpp, R. G., and Eady, H. R. (1956). “Some measurements of interaural time difference thresholds.” *J. Acoust. Soc. Am.* **28**, 859–860.

- Koay, G., Bitter, K. S., Heffner, H. E., and Heffner, R. S. (2002a). "Hearing in American leaf-nosed bats. I: *Phyllostomus hastatus*," Hear. Res. **171**, 96–102.
- Koay, G., Heffner, R. S., and Heffner, H. E. (2002b). "Behavioral audiograms of homozygous medJ mutant mice with sodium channel deficiency and their unaffected littermates," Hear. Res. **171**, 111–118.
- Koay, G., Kearns, D., Heffner, H. E., and Heffner, R. S. (1998). "Passive sound localization ability of the Big brown bat (*Eptesicus fuscus*)," Hear. Res. **119**, 37–48.
- Kuhn, G. F. (1997). "Model for the interaural time differences in the azimuthal plane," J. Acoust. Soc. Am. **62**, 157–167.
- Kuwabara, N., and Bhatnagar, K. P. (1999). "The superior olfactory complex of the vampire bat, *Desmodus rotundus* (Chiroptera: Phyllostomidae)," Acta Chiropterologica **1**, 81–92.
- Manske, U., and Schmidt, U. (1976). "Visual acuity of the vampire bat, *Desmodus rotundus*, and its dependence upon light intensity," Z. Tierpsychol. **42**, 215–221.
- Masterton, R. B., Thompson, G. C., Bechtold, J. K., and RoBards, M. J. (1975). "Neuroanatomical basis of binaural phase-difference analysis for sound localization: A comparative study," J. Comp. Physiol. Psychol. **89**, 379–386.
- McFadden, D., and Passanen, E. G. (1976). Lateralization at high frequencies based on interaural time differences. J. Acoust. Soc. Am. **59**, 634–639.
- Mills, A. W. (1958). "On the minimum audible angle," J. Acoust. Soc. Am. **30**, 237–246.
- Mills, A. W. (1972). "Auditory localization," in *Foundations of Modern Auditory Theory*, edited by J. V. Tobias (Academic, New York), Vol. 2, pp. 303–348.
- Obrist, M. K., Fenton, M. B., Eger, J. J., and Schlegel, P. A. (1993). "What ears do for bats: A comparative study of pinna sound pressure transformation in Chiroptera," J. Exp. Biol. **180**, 119–152.
- Perrott, D. R. (1969). "Role of signal onset in sound localization," J. Acoust. Soc. Am. **45**, 436–445.
- Pettigrew, J. D., Dreher, B., Hopkins, C. S., McCall, M. J., and Brown, M. (1988). "Peak density and distribution of ganglion cells in the retinae of microchiropteran bats: Implications for visual acuity," Brain, Behav. Evol. **32**, 39–56.
- Plack, C. J. (2005). *The Sense of Hearing* (Routledge Press, Florence, KY).
- Schmidt, U. (1988). "Orientation and sensory functions in *Desmodus rotundus*," in *Natural History of Vampire Bats*, edited by A. M. Greenhall and U. Schmidt (CRC, Boca Raton, FL), pp. 143–166.
- Schwartz, I. R. (1992). "The superior olfactory complex and lateral lemniscal nuclei," in *The Mammalian Auditory Pathway: Neuroanatomy*, edited by D. B. Webster, A. N. Popper, and R. R. Fay (Springer, New York), pp. 117–167.
- Simmons, J. A., and Simmons, A. M. (2011). "Bats and frogs and animals in between: Evidence for a common central timing mechanism to extract periodicity pitch," J. Comp. Physiol. A **197**, 585–594.
- Skottun, B. C. (1998). "Sound localization and neurons," Nature **393**, 531.
- Wesolek, C. M., Koay, G., Heffner, R. S., and Heffner, H. E. (2010). "Laboratory rats (*Rattus norvegicus*) do not use binaural phase differences to localize sound," Hear. Res. **265**, 54–62.
- Zook, J. M., and Casseday, J. H. (1980). "Identification of auditory centers in lower brain stem of two species of echolocating bats: Evidence from injection of horseradish peroxidase into inferior colliculus," in *Proceedings of the Fifth International Bat Research Conference*, edited by D. E. Wilson and A. L. Gardner (Texas Tech Press, Lubbock, TX), pp. 51–59.