Use of binaural cues for sound localization in large and small non-echolocating bats: *Eidolon helvum* and *Cynopterus brachyotis*

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The authors determined the ability of two old-world non-echolocating bats, *Eidolon helvum* and *Cynopterus brachyotis*, to use binaural time and intensity difference cues for localization. The bats were trained to localize pure tones throughout most of their hearing range from loudspeakers located 30° to the left and right of midline. Both species easily localized high frequency tones, indicating they could use the interaural intensity difference cue. However, neither was able to localize low frequency tones even when the tones were amplitude modulated thereby indicating that they could not use ongoing phase difference cues. The authors now know of eight mammals that do not use binaural phase cues for localization, and some possible reasons for this inability are explored.

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I. INTRODUCTION

The sense of hearing allows animals to detect and locate other animals even if they remain unseen. Despite the adaptive value of such universal functions, hearing and sound-localization abilities vary widely among different species, and these differences can provide insight into the evolution and physiology of the auditory sense. For example, hearing ranges vary so widely that even within mammals, there are species that live in entirely different auditory worlds—pocket gophers, blind mole rats, and elephants hear few or, in some cases, no sounds in common with many bats and some rodents, such as spiny mice and house mice. These differences are explained almost entirely by the adaptive value of high frequency hearing for sound localization in small species (Heffner and Heffner, 1998). Similarly, the ability to localize sound sources varies from the highly acute (e.g., 1°–2° resolution of humans, elephants, and pigs) to the near inability to localize sources separated by as much as 180° seen in some subterranean species (Heffner and Heffner, 1992b, 1993; Heffner *et al.*, 2008). We can account for much of this variation in sound localization by the requirement for more precision in species that must foveate a sound source compared to those whose vision is spread into a broad horizontal streak (Heffner and Heffner, 1992b; Heffner *et al.*, 2007).

Mammals also differ in the binaural cues they use for sound localization, but the amount of variation in this feature, and its likely basis, remain poorly understood as too few species have been examined. There are two binaural cues to locus in the azimuthal plane, namely, a low frequency cue that uses interaural time differences and a high frequency cue that uses interaural intensity or spectral differences. Many of the mammals examined so far use both binaural cues, but others rely on only one (Heffner and Heffner, 2003). In particular, several species of rodents and bats, as well as hedgehogs, have been found to be unable to use interaural phase differences (Heffner and Heffner, 1992a; Heffner *et al.*, 2010; Koay *et al.*, 1998b; Masterton *et al.*, 1975; Wesolek *et al.*, 2010). Although these species are all relatively small, suggesting the possibility that some interaural distances may be just too small to provide useful time differences, other small mammals, including two species of bats, Jamaican fruit bats (*Artibeus jamaicensis*) and Egyptian fruit bats (*Rousettus aegyptiacus*), retain the ability to localize sound sources using the interaural time difference cue despite their small size (Heffner *et al.*, 1999, 2001a). So far there is no obvious factor that distinguishes between species that do, and those that do not, use time cues.

To expand the sample of species on which to base our understanding of the use of binaural locus cues, we examined the ability to use the interaural time and intensity difference cues in two species of non-echolocating bats in the family Pteropodidae: The Straw-colored fruit bat (*Eidolon helvum*), a large (300 g) species native to Africa, and the Dog-faced fruit bat (*Cynopterus brachyotis*), a small (35 g) species found throughout southern Asia. Both species are abundant, and despite their contribution to pollination and seed dispersal, are considered crop pests. Earlier studies determined that their passive sound-localization acuity is near the mean for mammals (approximately 12°, Heffner *et al.*, 2007), with *E. helvum* having a threshold of 11.7°, and the smaller *C. brachyotis* having a threshold of 10.5° (Heffner *et al.*, 2008).

II. METHODS

Two individuals of each species were tested for their ability to localize brief pure-tone pips ranging from 4 to 32 kHz (*E. helvum*) or from 5.6 to 50 kHz (*C. brachyotis*). Pure-tone localization allows us to assess the use of the binaural locus cues without the technical difficulties inherent in using

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headphones in small wild animals that must remain awake and unrestrained for behavioral testing (Masterton *et al.*, 1975; Mills, 1972). Low frequency tones permit comparison of the arrival time of corresponding parts of a sine wave at the two ears (the phase difference cue); with no attenuation by the head and pinnae, they present no interaural intensity difference cue (e.g., Plack, 2005). This phase difference cue becomes ambiguous at high frequencies when more than one half cycle of the tone occurs during the time it takes for the sound to travel from one ear to the other. Above this "frequency of ambiguity," interaural intensity differences, if present, are the only binaural cues available for localization in the azimuthal plane.

It should be noted that neither of these bats hears the lower frequencies (below 1 kHz) that most species find easiest to localize using ongoing time cues. To test whether they retain the use of time cues despite their restricted hearing range, an additional basis for a time analysis was provided. A pure tone that was easily heard but that was not localizable (4 kHz for *E. helvum* and 5.6 kHz for *C. brachyotis*) was amplitude modulated at rates of 500 and 1000 Hz to permit extraction of ongoing time differences from the envelope of the signal.

Tone-localization performance was assessed throughout the bats' hearing ranges, within the constraints of producing a pure tone 50 dB SL (sensation level) for each species for good audibility (see Heffner *et al.*, 2006 for audiograms). We used the same conditioned avoidance procedure used to test many other mammals in which the bats were trained to avoid a mild electric shock by breaking contact with the reward spout whenever a tone was presented from the left while continuing to drink from the spout when a tone came from the right.

A. Subjects

Two *Eidolon helvum* (bat A, female, 266 g, and approximately 3 years old; and bat B, male, 320 g, and approximately 9 years old) and two *Cynopterus brachyotis* (bat A, male, 38 g; and bat B, female, 40 g; both approximately 2 years old) were tested. All four individuals had been tested previously to determine their noise-localization acuity and behavioral audiograms (Heffner *et al.*, 2006, 2008). The mean maximum functional interaural distance (i.e., the time required for a sound to travel around the head from one auditory meatus to the other) was 145 μs for *E. helvum* and 86 μs for *C. brachyotis*. The bat colonies were maintained on a diet of mixed fruit (Barnard, 1995) and a dietary supplement (Lubee fruit bat supplement). While on test, the bats were housed individually in wood and plastic mesh cages ($48 \times 39 \times 95$ cm^3). They had free access to water and earned their food in the test sessions, except for occasional supplements to maintain healthy bodyweights. All bats were captive born and on loan from the Lubee Bat Conservancy. These experiments were carried out with the approval of 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; $2.55 \times 2.75 \times 2.05$ m^3), the walls and ceiling of which were lined with acoustic foam. The equipment for stimulus generation and behavioral measurement was located outside the chamber and the bats were observed via closed-circuit television.

Sounds were presented through loudspeakers at ear level, mounted on a perimeter bar (102 cm radius and 101 cm height) and centered on the position occupied by an animal's head while it was drinking from the spout. The bats were tested in custom-built wire mesh cages. So that each species could maneuver easily, the test cage for *E. helvum* measured $50 \times 30 \times 50$ cm^3 and was constructed of 1-in. (2.5-cm) mesh, and the cage for *C. brachyotis* was $37 \times 22 \times 23$ cm^3, constructed of 0.5-in. (1.26-cm) mesh. The bats climbed onto a small raised platform in the middle of the test cage to reach a reward spout placed in front of the platform. This configuration minimized acoustic obstructions between the bats and the loudspeakers. The platform was covered with a dampened carpet to provide traction and electrical contact between the bat and reward spout. When the bat licked the reward spout, a steady trickle of fruit juice was dispensed using a syringe pump. The pump was housed in a foam-lined box and placed in the back of the test chamber to eliminate dispenser noise. The fruit juice consisted of a mixture of cantaloupe and pear juice with the dietary supplement. Requiring the bat to steadily lick the reward spout for fruit juice served to maintain its head in a fixed position in the sound field. A shock generator was also connected between the reward spout and platform; the shock level was adjusted for each individual to the lowest intensity that produced a reliable avoidance response (breaking electrical contact with the spout). The bats did not develop a fear of the spout, as they readily returned to it after the shock. A 25-W shock-indicator light, placed below the cage, was turned on and off concurrently with the shock to signal a successful avoidance and indicate when it was safe to resume licking the spout. (See Heffner *et al.*, 2006, 2008 for detailed descriptions of the two test cages.)

C. Acoustical apparatus

Pure tones were generated using a digital tone generator (Zonic A and D 3525). The tones were randomly attenuated over a 3.5-dB range from one trial to the next (Coulbourn S85-08 programmable attenuator) to eliminate responses based on any slight intensity imbalance between speakers. Because tone localization is difficult for most species, the tones were pulsed (100 ms on and 900 ms off) for two pulses, 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 two loudspeakers (Panasonic EAS-10TH400C). The acoustic signal at the location of a listening bat was analyzed for overtones using a spectrum analyzer (Zonic A and D 3525), and any harmonics in the acoustic signal were at least 40 dB below the fundamental frequency and below the animal's detection threshold. Tones were calibrated at the beginning and end of each test session.

Testing was conducted with the loudspeakers placed 60° apart (30° to the left and right of midline) for both species. *E. helvum* was tested at 4, 5.6, 8, 10, 12.5, 20, 25, and 32 kHz. For *C. brachyotis*, frequencies tested were 5.6, 8, 12.5, 16, 32, and 50 kHz. For each species, the tone pulses were presented at 50 dB above the mean absolute threshold at that frequency (Heffner *et al.*, 2006). Furthermore, all frequencies were shaped using a 10-ms rise-decay time to avoid onset and offset transients.

Additional tests were conducted with a 4-kHz (*E. helvum*) or 5.6-kHz (*C. brachyotis*) carrier tone that was sinusoidally amplitude modulated (Krohn-Hite 2400 AM/FM phase lock generator) at 0.5 and 1 kHz (100% modulation depth) to provide an ongoing binaural time difference cue in the envelope of the signal. Previous research has shown that a tone that is too high in frequency to permit use of the binaural phase difference cue, yet too low to generate an interaural intensity difference, can still be localized if it is amplitude modulated (Heffner *et al.*, 2001a). It appears that animals can extract a binaural time cue from the signal's envelope even when they cannot extract it from the carrier.

D. Sound level measurement

The sound pressure levels (SPLs) of the stimuli (SPL re 20 $\mu N/m^2$) were measured and the left and right loudspeakers were equated daily with a 1/4-in. (0.64 cm) microphone (Brüel and Kjaer 4135, with correction for protection grid), preamplifier (Brüel and Kjaer 2619), measuring amplifier (Brüel and Kjaer 2608), and spectrum analyzer (Zonic A and D 3525) to permit detection of any harmonics that might be present. This 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 toward a loudspeaker (0° incidence).

E. Behavioral procedure

1. Training

All four bats had previously been tested to determine their noise-localization thresholds, and generalized to the tone-localization task without difficulty. They were required to lick the reward spout in the presence of two 100 ms tone bursts (900 ms interpulse interval), presented from a loudspeaker located 30° to their right, and then to break contact with the spout (a "detection response") whenever the tone bursts were presented 30° to their left. Breaking contact allowed the bats to avoid a mild electric shock (0.5 s), delivered from the spout 2 s after left signal onset, while also indicating that they had perceived the shift in locus. The light bulb underneath the cage was turned on concurrently with shock as feedback for a successful avoidance (since, in those cases, the bat received no shock) and permitted the animals to distinguish between successful avoidance of a shock and false alarms (i.e., breaking contact when the signal was on the right).

2. Testing

Test sessions consisted of a series of 2-s trials (100 ms on and 900 ms off, for two pulses) separated by 1.5-s intertrial intervals. Thus every 3.5 s the bats received two brief tone pulses and decided whether to break contact or to continue drinking after the tone burst. The animal's response was defined as the duration of contact with the spout during the last 150 ms of each 2-s trial. If the animal 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 side and as a "false alarm" if it had come from the right. If the bat was not in contact with the spout during the 1 s preceding a trial, data from that trial were not recorded but the trial continued as usual. Thus, any trials in which a bat was grooming or otherwise not engaged in the task were discarded.

Each trial had a 22% probability of containing a left signal. The sequence of left-right trials was quasi-random (for details see Heffner and Heffner, 1995). Hit and false alarm rates were determined for each block of approximately 7–9 left trials and 28–36 associated right trials. 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 ranges from 0 (no hits) to 1 (100% hit rate with no false alarms), and proportionately reduces the hit rate by the false alarm rate observed for each block of trials, rather than by the average false alarm rate for the entire session. This results in a more precise performance measure for a specific block of trials as false alarm rates often vary within a session depending on the discriminability of the stimulus and the animal's level of motivation.

Testing was carried out on 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 to verify that it remained sufficiently motivated. Each frequency was tested for at least three sessions for an average of 90–100 left trials. The top 50% of the trial blocks were then averaged to represent the best overall performance the animals were capable of sustaining.

III. RESULTS

A. Pure-tone localization

Figures 1 and 2 illustrate the tone-localization performances of *E. helvum* and *C. brachyotis*, as well as the estimated availability of the two binaural locus cues. At an angle of ±30°, the phase cue is estimated to become physically ambiguous at frequencies higher than 7.15 kHz for *E. helvum* (maximum interaural delay of 145 μs). For *C. brachyotis*, the phase cue becomes ambiguous above about 12.06 kHz (maximum interaural delay of 86 μs); at higher frequencies interaural intensity differences must be used for localization. (For a detailed discussion of phase ambiguity, see Saberi *et al.*, 1998; Woodworth and Schlosberg, 1954.) However, interaural intensity differences are strongest only at frequencies with wavelengths shorter than the head diameter (or shorter than pinna dimensions for species with pinnae larger

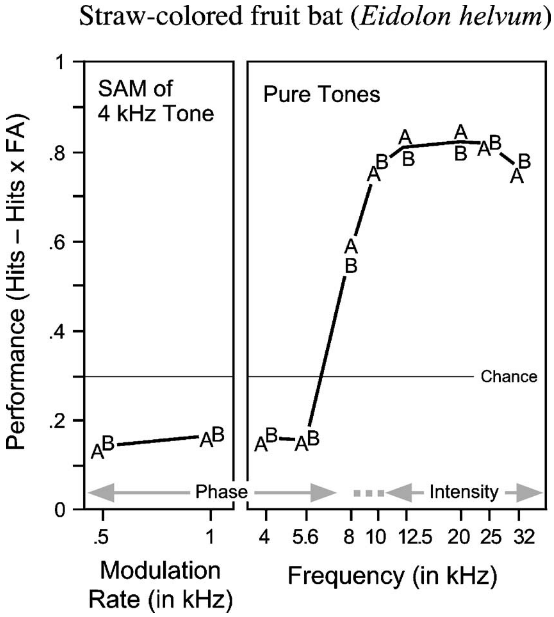

FIG. 1. Sound-localization performance for two *E. helvum* as a function of the frequency of a pure-tone stimulus and the modulation rate of a 4-kHz tone (two pulses of 100 ms duration and 900 ms interpulse interval) at a fixed angle of ±30° azimuth. Letters represent two individual bats. Availability of the interaural phase difference and intensity difference cues is indicated by the gray arrows along the frequency axis. Note that the bats perform well only at frequencies for which the intensity cue is expected to be available and performance falls to chance when they must rely on the phase cue.

than the head). For *E. helvum*, with a head diameter of approximately 2.9 cm, intensity differences at the two ears should be present above 10.8 kHz, and for *C. brachyotis* with a head diameter of 1.9 cm, intensity differences should be present above 18.27 kHz. However, measurable head shadowing can occur even two octaves lower (where wavelengths are four times as long as the head diameter), perhaps accounting for the residual but poor performance at frequencies just below those at which interaural intensity differences are calculated to begin to diminish (for a discussion of sound diffraction, see Christensen-Dalsgaard, 2005).

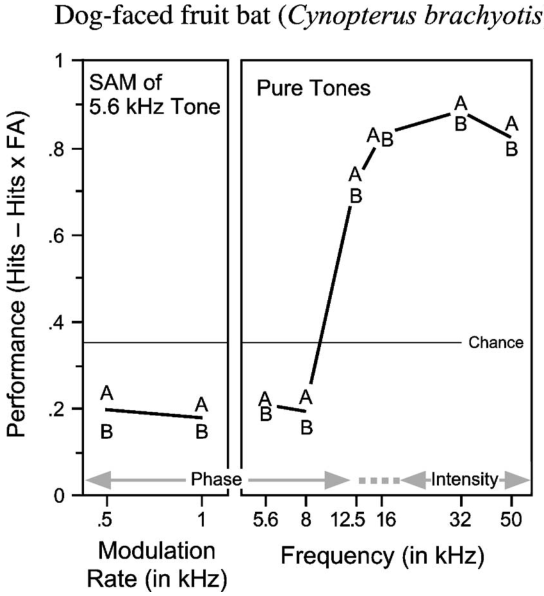

FIG. 2. Sound-localization performance for two *C. brachyotis* as a function of the frequency of a pure-tone stimulus and the modulation rate of a 5.6-kHz tone (two pulses of 100 ms duration and 900 ms interpulse interval) at a fixed angle of ±30° azimuth. Letters represent two individual bats. Availability of the interaural phase difference and intensity difference cues is indicated by the gray arrows along the frequency axis. Note that the bats perform well only at frequencies for which the intensity cue is expected to be available and performance falls to chance when they must rely on the phase cue.

As Fig. 1 illustrates, the two *E. helvum* showed good agreement in the tone-localization task, with good performance at frequencies of 10 kHz and higher, indicating good use of interaural intensity differences for localization. As sound shadowing by the head and pinnae began to diminish below 10.8 kHz, performance began to fall with both individuals performing less successfully at 8 kHz (albeit still above chance). However, at the two lower frequencies of 5.6 and 4 kHz, performance was at chance indicating that, although the interaural phase difference cue was available, the bats could not make use of that cue for localizing brief sounds. Testing could not be carried out with lower frequency pure tones because the bats' insensitivity below 4 kHz did not permit undistorted production of pure tones at sufficient intensities. However, as noted below, lower frequencies can be presented as envelope fluctuations in amplitude modulated sounds.

Figure 2 illustrates the tone-localization performance of *C. brachyotis*. Here, too, both performed well at high frequencies indicating good ability to localize using the interaural intensity difference cue. As the head-shadowing became less effective below 18 kHz, performance worsened for both individuals but still remained above chance at 12.5 kHz. At 8 kHz and below, performance of both bats declined sharply to chance.

By failing to localize low frequencies, while at the same time showing good motivation and performance at high frequencies, the bats demonstrated that they cannot use interaural phase differences to localize pure tones. Motivation cannot explain the decline in performance for either species because good performance returned rapidly within a test session when high frequencies were presented as part of a broadband signal.

B. Sinusoidal amplitude modulation

To further explore the ability of *E. helvum* and *C. brachyotis* to use binaural time differences, we introduced a time cue in the envelope of the signal by amplitude modulating the tone. For *E. helvum* we used a 4-kHz carrier tone and for *C. brachyotis* we used a 5.6-kHz carrier tone, neither of which could be localized by those species. The tones were modulated at 500 Hz and 1 kHz (frequencies easily localized by species that can use time cues; e.g., Heffner and Heffner, 1987; Heffner *et al.*, 1999, 2001a). Amplitude modulation also resulted in the production of side lobes, i.e., tones of frequencies equal to the carrier frequency plus and minus the modulation rate. Modulation rates were chosen so that the higher frequency side lobes remained too low to be localized using an intensity difference. For example, modulating the 4-kHz tone at 1 kHz for *E. helvum* produced side lobes of 3 and 5 kHz. Modulating the 5.6-kHz tone at 1 kHz for *C. brachyotis* produced side lobes at 4.6 and 6.6 kHz. As shown in Figs. 1 and 2, the modulation of the envelope and the presence of side lobes did not improve the localization performance for either species as both continued to perform at chance. Thus, neither species was able to localize using interaural time differences in the carrier wave or the envelope of the signal.

IV. DISCUSSION

Both *E. helvum* and *C. brachyotis* localized high frequencies easily, performing approximately as well with pure tones as they had with broadband noise at the same angle (Heffner *et al.*, 2008) thereby demonstrating their ability to use interaural intensity differences for localization. This good localization of high frequencies contrasted sharply with their inability to localize lower frequencies that require the use of the interaural phase difference cue. For wavelengths longer than the head diameter—approximately 10.8 kHz for *E. helvum*, and 18.3 kHz for *C. brachyotis*—the head becomes less effective at blocking sound waves so that differences between the two ears become smaller (indicated by the dashed gray line in Figs. 1 and 2). It was below these frequencies that the bats' performances fell sharply—within an octave performance fell to chance and did not recover at lower frequencies for either species. Taken together, these results indicate that both of these non-echolocating bats use binaural intensity difference cues but not ongoing phase difference cues for sound localization.

The demonstration that the bats are unable to use binaural phase differences to localize sound indicates that they are unable to use binaural time cues, at least at low frequencies. Although we cannot rule out the possibility that the bats were using binaural time cues to localize high frequencies (e.g., in the envelope of a high frequency signal), we can think of no reason why they would retain the ability to use time differences to localize high frequencies where binaural intensity differences are present and relinquish the time cue at low frequencies where no other localization cue is available. However, before concluding that these bats are completely unable to use binaural time cues to localize sound, it is necessary to consider the transient interaural differences that exist at a sound's onset, i.e., the transient onset cue.

When a sound is first turned on, there is a transient interaural difference that begins when the sound reaches threshold at the near ear and ends when it reaches full intensity at the far ear. The observation that the bats could not localize low frequencies indicates that they could not make use of the onset cue in that frequency range, probably because the onset cue is not a strong cue to locus (e.g., Krahe *et al.*, 2000; Perrott, 1969). Although we cannot rule out the possibility that the bats nevertheless used the onset cue to localize the high frequencies, whether or not they did so does not address the question of whether they were using binaural time differences to localize those frequencies. This is because the onset cue seems to be a transient interaural intensity difference cue and not a time cue. This was demonstrated by a now classic series of experiments on the role of onset in sound localization by Elfner and his colleagues (Elfner and Tomsic, 1968; Perrott, 1969). Specifically, they noted that changing the rise time of a signal has opposite effects on the size of the arrival time difference and the size of the transient interaural intensity difference. Increasing the rise time of a signal (i.e., turning it on more slowly) increases the difference in the arrival time of a sound at the two ears, but decreases the transient interaural intensity difference. They addressed the question of whether the auditory system analyzes the onset cue as a time or intensity difference by determining the effect of rise time on sound-localization ability. They found that increasing the rise time of a signal decreased the ability of subjects to use to the onset cue to either localize or lateralize sound. Thus *performance declined with the decrease in the intensity difference* rather than improved as would be expected if the subjects were relying on the increasing time difference. This remarkable result led them to conclude that the interaural differences in signal onset is best viewed as a short-duration intensity difference (Elfner and Tomsic, 1968; Perrott, 1969). Their conclusions are not limited to humans because the same effect of signal onset has recently been observed in rats (Wesolek *et al.*, 2010). Consequently, we conclude that animals that cannot use binaural phase cues to localize sound are also unlikely to use the delay in envelope onset. Finally, the finding that the onset delay was present for the two species in this report at all frequencies tested, yet the bats could localize only those sounds for which an intensity difference was present, is most parsimoniously explained by their inability to use the ongoing phase difference cue.

We should note that there is another localization phenomenon in which the difference in the arrival time of a signal at the two ears plays a role. This is the precedence effect in which the same sound presented from two speakers is localized to the speaker that is turned on first, regardless of the intensity of the second speaker (Wallach *et al.*, 1949). The precedence effect, however, differs from the onset cue in that there are two or more sound sources, and it operates over a range of milliseconds whereas the onset cue for locus pertains to a single sound source and is effective in the microsecond range. Although the precedence effect is commonly viewed as a phenomenon dependent upon time because delayed sounds—echoes in the natural environment—are suppressed (e.g., Devore *et al.*, 2009), it can also be viewed as depending upon an interaural intensity difference (Elfner and Tomsic, 1968). Thus, the response of an animal (or its neurons) to the long delays characteristic of precedence-effect stimuli may not necessarily indicate that the animal is able to process interaural time differences, especially if that animal is unable to process time cues in other stimuli such as those delayed in phase.

A. Use of binaural locus cues by bats

In addition to the two non-echolocating species reported here, five echolocating bats have been tested for their ability to use binaural locus cues: Egyptian fruit bat, Jamaican fruit bat, Short-tailed fruit bat, Greater spear-nosed bat, and Big brown bat (for references, see Table I). As summarized in

TABLE I. Range of frequencies for which the binaural phase cue is physically available to mammals with restricted low frequency hearing. Despite the physical availability of the binaural time cue to all the species listed, only two are able to use it. This suggests that we cannot attribute the failure to use this cue to any restriction of its availability.

Species	Lowest frequency audible at 60 dB in kHz	Frequency of phase ambiguity in kHz	Available range of interaural phase difference cue (in octaves)[a]	Use binaural time cue
Long-eared hedgehog *Hemiechinus auritus*	2.4	6.21	1.4	No[b]
Egyptian fruit bat *Rousettus aegyptiacus*	2.25	7.68	1.8	Yes[c]
Jamaican fruit bat *Artibeus jamaicensis*	2.8	11.5	2.0	Yes[d]
Dog-faced fruit bat (non-echolocator) *Cynopterus brachyotis*	2.79	12.06	2.1	No
Short-tailed fruit bat *Carollia perspicillata*	5.2	22.06	2.1	No[e]
Straw-colored fruit bat (non-echolocator) *Eidolon helvum*	1.46	7.15	2.3	No
Greater spear-nosed bat *Phyllostomus hastatus*	1.77	9.60	2.4	No[e]
Big brown bat *Eptesicus fuscus*	3.65	20.74	2.5	No[f]
Domestic mouse *Mus musculus*	2.35	16.98	2.9	No[g]
Norway rat *Rattus norvegicus*	0.520	6.91	3.7	No[h]

[a] The phase difference cue is considered to become available at the lowest frequency audible at 60 dB SPL and extend to the frequency at which the cue becomes physically ambiguous. Here we use an angle of 30° from midline, but even with the slightly narrower range of availability with larger angles, the argument that the cue is available to these species over a substantial frequency range remains the same.

[b] Masterton *et al.*, 1975.

[c] Koay *et al.*, 1998a.

[d] Heffner *et al.*, 2001a.

[e] Heffner *et al.*, 2010.

[f] Koay *et al.*, 1998b.

[g] Heffner *et al.*, 2001c.

[h] Wesolek *et al.*, 2010.

Fig. 3, all bats localize high frequencies with their accompanying interaural intensity differences, but not all are able to localize lower frequencies that require reliance on interaural phase differences. Like the two non-echolocating species, three echolocating species, Big brown bats (*Eptesicus fuscus*), Greater spear-nosed bats (*Phyllostomus hastatus*), and Short-tailed fruit bats (*Carollia perspicillata*), also do not use the binaural time cue in the form of phase differences (Heffner *et al.*, 2010; Koay *et al.*, 1998b). On the other hand, the echolocating Jamaican fruit bat (*Artibeus jamaicensis*) and the Egyptian fruit bat (*Rousettus aegyptiacus*) that uses simple tongue clicks for echolocating were able to use interaural phase differences with both low frequency pure tones and amplitude modulated envelopes of an unlocalizable carrier tone (Heffner *et al.*, 1999, 2001a). Thus, the ability to use time difference cues does not distinguish echolocating from non-echolocating bats.

B. Use of binaural time cues by small mammals

For many years we believed that all mammals used both the binaural time and intensity difference locus cues, just as humans do (Stevens and Newman, 1936). The first species reported to be unable to use both cues was the Long-eared hedgehog (*Hemiechinus auritus*) that, like the species reported here, could not use binaural phase cues (Masterton *et al.*, 1975). Since then altogether eight species in three different orders have been discovered that lack the ability to use interaural phase differences. Other mammals were later found that are unable to use the binaural intensity difference cue (Heffner and Heffner, 2003), further indicating that the binaural cues are subject to independent selective pressure and that a comparative analysis of the use of locus cues will increase our understanding of the evolution of sound localization and its underlying mechanisms.

There are several plausible reasons that animals might not use phase differences for sound localization: (1) They do not hear the low frequencies that require the use of binaural time cues, (2) they do not need to localize sound accurately, or (3) their heads may be too small to generate useful time cues. We address these possibilities in the light of the two additional cases reported here.

1. Use of time cues and low frequency hearing

Mammals fall into two groups, with most having good low frequency hearing that extends below about 125 Hz (at a level of 60 dB SPL or less). However, about a third of mammals examined so far do not hear below about 500 Hz, and there are virtually no intermediate species in this bimodal distribution (Heffner *et al.*, 2001b). Thus, the ability to hear low frequencies is not normally distributed among mammals, and it is reasonable to ask whether the simple ability to hear the low frequencies that form the basis for the time cue can explain the differences in the ability of mammals to use that cue. However, this does not seem to be the case.

Low frequency hearing may be sufficient to support use of the low frequency locus cues since so far all species with good low frequency hearing can also use interaural phase differences to localize (subterranean mammals, being unable to localize, are potential exceptions, e.g., Heffner and Heffner, 1993). However, good low frequency hearing is not necessary. Although most of the species with restricted low frequency hearing do not use phase delays, two species do: the Jamaican fruit bat (*Artibeus jamaicensis*) and the Egyptian fruit bat (*Rousettus aegyptiacus*). Further, those species

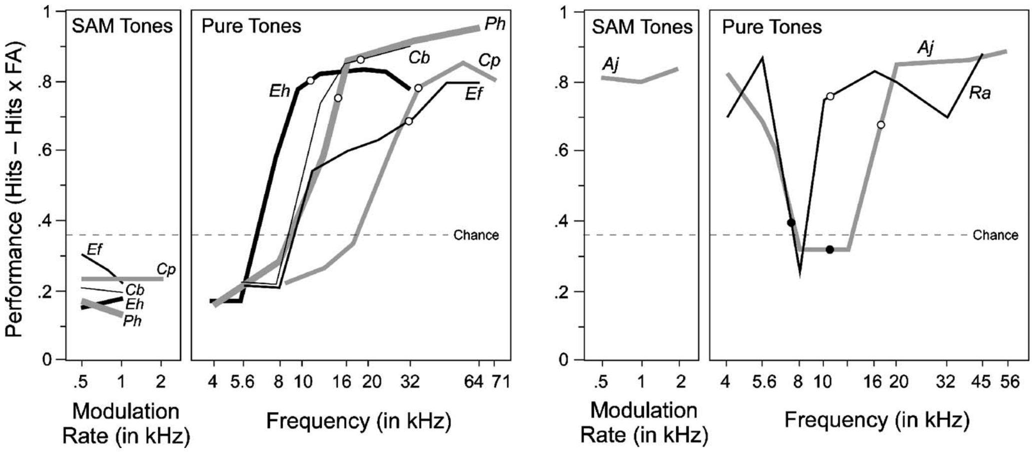

FIG. 3. Sound-localization performance as a function of the frequency of either a pure tone or the modulation frequency of an amplitude modulated carrier tone. The left panel depicts five bats that could not localize frequencies below which the head does not act as an effective sound shadow to produce an interaural intensity difference—the calculated value of this frequency for each species is indicated by a small open circle and performances decline below that point. The right panel depicts two bats that localize high frequencies as well as low frequencies that rely on interaural phase differences. Note that for both of these species there are frequencies at which performance fell to chance. These frequencies appear to be those for which neither an intensity difference nor an unambiguous phase difference is available. (Phase differences are ambiguous at wavelengths short enough that more than one half cycle occurs during the time it takes for the sound to travel from one ear to the other; that point is indicated for *Artibeus jamaicensis* and *Rousettus aegyptiacus* by the closed circles. Thus the phase difference cue is physically ambiguous for these species at higher frequencies.) For references, see Table I.

that have relinquished the use of phase differences have done so even though the cue is physically available to them over a considerable range of frequencies, as shown in Table I. Indeed, two of the species with the more limited availability of the cue are those that nevertheless continue to use it. Thus restricted low frequency hearing and limited cue availability are not good explanations for foregoing the use of phase difference time cues for localization.

2. Use of time cues and localization acuity

It might be considered that the species that do not use the binaural time cues gave up that cue because they are simply poor localizers and do not need the accuracy that might be provided by the robust time cues. However, the species that do use time cues have localization acuity within the range of similar-sized species that do not use the cues (Heffner *et al.*, 2010). Indeed, among the species that do not use time cues, many have better localization thresholds (including the 10.5°–12° thresholds of the two species reported here, Heffner *et al.*, 2008) than larger species that do use time cues such as chinchilla, gerbil, horse, and cattle (Heffner and Heffner, 1984, 1988, 1992c; Heffner *et al.*, 1994). Thus, loss of the time cue does not necessarily compromise sound-localization acuity and the idea that animals give up the binaural time cue because they have less need for accurate localization is not supported.

3. Use of time cues with short interaural delays

All the mammals that do not use binaural time cues are relatively small species. Thus, it might be proposed that their ears are so close together that the brief time differences available (e.g., less than 100 μs) can provide only an approximate indication of locus and are thus not very useful for localization. If so, then one would expect that animals with head sizes below some minimum would not use binaural time differences. To see if this is the case, we compared the maximum interaural time delay in the eight species unable to use the phase difference cue with that of the seven smallest species currently known to use the cue. As shown in Fig. 4, there is considerable overlap between the two groups. Five of the species that *do* use the binaural phase difference cue

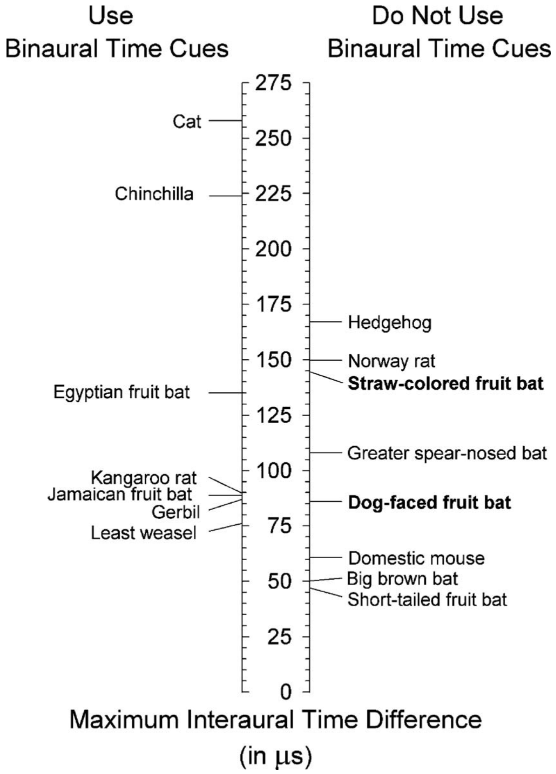

FIG. 4. Species that do use time cues compared to those that do not. Species are ranked according to the magnitude of their interaural time difference (the amount of time it would take sound to travel around the head from the opening of one auditory meatus to the other). Note that four of the species that do use time cues are quite small, but none of the species with interaural time differences shorter than 75 μs use time cues.

fall well within the range of species that *do not* use the cue. Thus, many species retain the use of interaural time delays for localization despite the short delays available to them. Even so, it remains possible that below some very small head size, perhaps 75 μs, no mammal uses time cues. Behavioral tests with additional species in this size range will be of interest in this regard.

C. Neural responses to interaural delays in small mammals

Neural responses to interaural delays have been recorded in both Norway rats and in some small bats, but it is noteworthy that those responses show little variation at delays within the range naturally available to these species (e.g., Fuzessery, 1997; Kelly and Phillips, 1991). Instead, the delays that elicit responses in the auditory system are often much longer and in the range of delays generated by echoes, leading some investigators to propose that, in small species, responses to long interaural delays may be involved in suppressing the echoes common in natural environments rather than for use in sound localization (Grothe, 2000; Grothe and Park, 2000). Our behavioral studies with bats and small rodents show that many species are consistent with this conclusion in that they do not use time cues for localization. However, the small species that do use time cues to localize sound (Fig. 4) demonstrate that it must be possible to circumvent any limitations attributable to small head size or neural timing mechanisms. Moreover, this has happened in the evolution of more than one mammalian lineage. Recordings of the neural responses to time delays in some of the small species that *do* use time cues might reveal that the mammalian auditory system is capable of discriminating smaller time differences than have so far been demonstrated.

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