Behavioral Neuroscience
1986, Vol. 100, No. 1, 93-103

0735-7044/86/$00.75

Localization of Tones by Horses: Use of Binaural Cues and the Role of the Superior Olivary Complex

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The ability of horses to use binaural time and intensity difference cues to localize sound was assessed in free-field localization tests by using pure tones. The animals were required to discriminate the locus of a single tone pip ranging in frequency from 250 Hz to 25 kHz emitted by loudspeakers located 30° to the left and right of the animals' midline (60° total separation). Three animals were tested with a two-choice procedure; 2 additional animals were tested with a conditioned avoidance procedure. All 5 animals were able to localize 250 Hz, 500 Hz, and 1 kHz but were completely unable to localize 2 kHz and above. Because the frequency of ambiguity for the binaural phase cue $\Delta\phi$ for horses in this test was calculated to be 1.5 kHz, these results indicate that horses can use binaural time differences in the form of $\Delta\phi$ but are unable to use binaural intensity differences. This finding was supported by an *un*conditioned orientation test involving 4 additional horses, which showed that horses correctly orient to a 500-Hz tone pip but not to an 8-kHz tone pip. Analysis of the superior olivary complex, the brain stem nucleus at which binaural interactions first take place, reveals that the lateral superior olive (LSO) is relatively small in the horse and lacks the laminar arrangement of bipolar cells characteristic of the LSO of most mammals that can use binaural ΔI.

For humans and most other mammals, there are two binaural cues for the localization of natural sounds. The first is called "Δt," which is the difference in the time of arrival of a sound at the two ears. The second is referred to as "Δfi," which is the difference in the frequency–intensity spectra of sounds reaching the two ears (Masterton & Diamond, 1973; Masterton, Thompson, Bechtold, & RoBards, 1975). Although the use of these two cues by humans has been known for some time (Rosenzweig, 1961), it is only recently that their use by other mammals has begun to be studied (e.g., Gourevitch, 1980).

The most common method of assessing the ability of animals to use binaural cues consists of requiring them to localize the source of single, brief tone pips of various frequencies (e.g., Masterton et al., 1975); that is, the localization of low-frequency tones is used as a measure of the ability to use the binaural phase-difference or $\Delta\phi$ cue, a special case of the Δt cue. The localization of high-frequency tones, on the other hand, is used as a measure of the ability to use binaural intensity differences or ΔI, a subset of the Δfi discrimination (Mills, 1972).

Although most mammals tested so far appear able to use both binaural cues, the results of tone localization tests have indicated that the ability of mammals to use these cues is neither uniform nor universal. In particular, the ability to use time cues varies significantly between species, and at least one animal, the hedgehog, appears to be totally unable to use $\Delta\phi$ (Masterton et al., 1975). Furthermore, this variation in sound localization ability has been linked to variation in the medial nucleus of the superior olivary complex, a brain stem auditory nucleus that appears to play a role in the analysis of $\Delta\phi$ (Masterton et al., 1975).

Because the ability to localize sound would seem to be such an important function of hearing, evidence of systematic variation in this ability suggests that an understanding of this variation may provide insight into the evolution of hearing in mammals. In particular, we might gain an understanding of the selective advantage of the ability to use the binaural time and intensity cues which are both so well developed in humans. Furthermore, variation in the use of the binaural locus cues may help interpret the variation in the anatomy and physiology of the auditory nuclei in the mammalian brain stem (cf. Masterton et al., 1975).

Though the use of $\Delta\phi$ has been shown to vary among mammals, previous studies have revealed only minor variation in the use of ΔI (R. Heffner & Heffner, 1982). In the present article, we present the results of behavioral tests which indicate that horses lack the ability to use ΔI to localize the source of a sound. In addition, an anatomical description of the superior olivary complex of the horse is presented as a part of a preliminary search for neuroanatomical correlates of the use of the ΔI cue.

This research was supported by Grant BNS-07391 from the National Science Foundation, National Institutes of Health Grants NS 17850 and HD 02528 to the Bureau of Child Research, University of Kansas, and Biomedical Sciences Support Grant R07037 to the University of Kansas.

We thank Richard Johnson, Director of the Kansas State Agriculture Experiment Station in Mound Valley, Kansas, and the Station staff for their assistance. We also thank the following for allowing us to use their animals: Kenneth McNickle, Ron McNickle, and Melvin Baker.

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General Method

The following experiments are divided into four sections. The first three sections concern the three behavioral tests used to determine the ability to localize tones. The fourth concerns the anatomical analysis of the superior olivary complex of the horse.

First, the ability of 3 horses to localize tones separated by an angle of 60° was determined by a two-choice procedure with water as a reward (cf. H. Heffner & Heffner, 1984). Second, in order to establish that the localization performance determined with the two-choice procedure was reliable, the 60° tone localization ability of 2 additional horses was determined by a shock avoidance procedure (cf. H. Heffner & Heffner, 1984). Third, the orientation response of 4 horses to high- and low-frequency tone pips (8 kHz and 0.5 kHz) was assessed to determine their unconditioned response to these sounds. Finally, the superior olivary complex of the horse was examined by using normal material in an attempt to relate the observed behavior to the neuroanatomy of the auditory system.

Experiment 1: Two-Choice Localization Test

Method

Subjects. Three horses between 21 and 23 months old (adolescent) were used in this test: a 340-kg quarterhorse gelding (Horse A), a 350-kg Appaloosa mare (Horse B), and a 260-kg Welsh pony × quarterhorse gelding (Horse C). All 3 animals had been used in previous absolute threshold and sound localization tests (R. Heffner

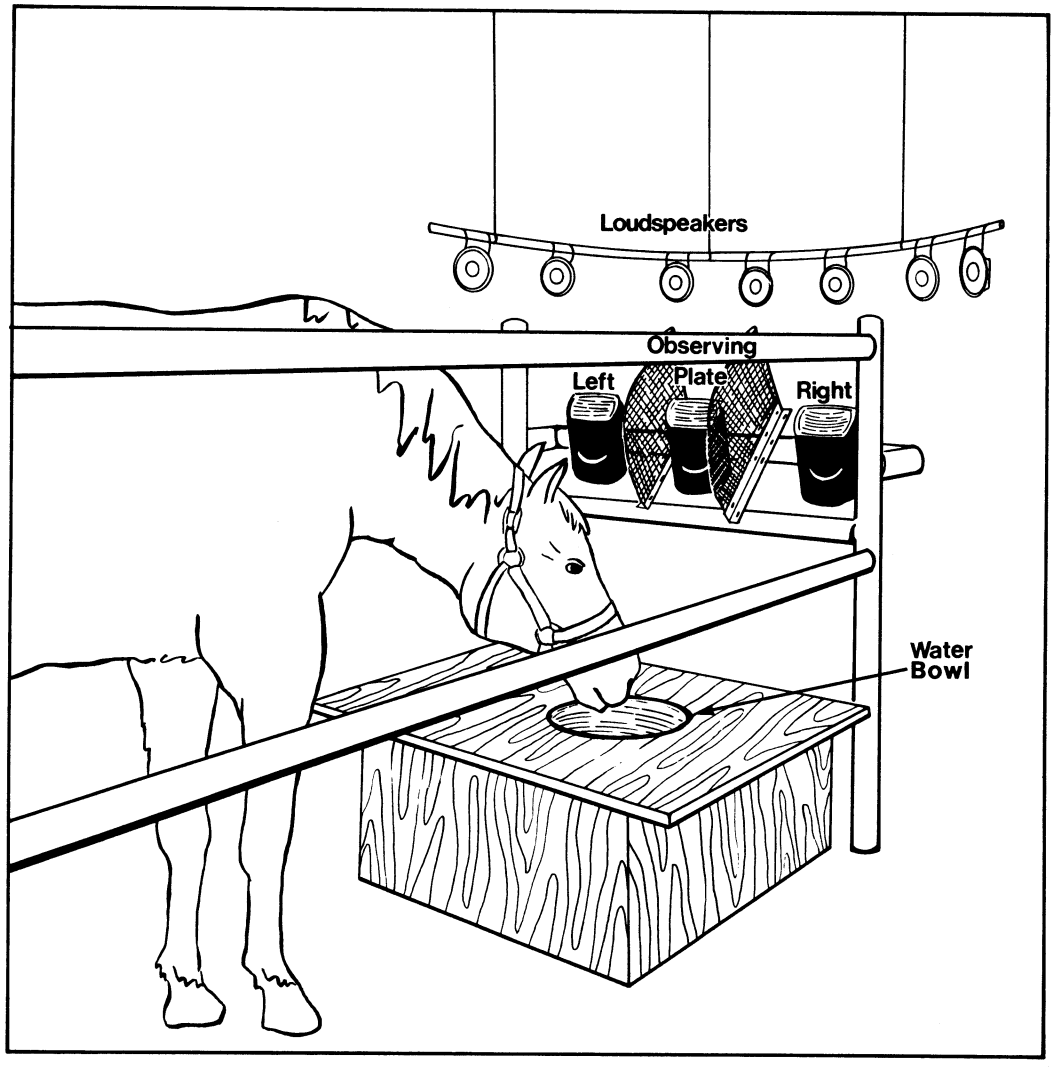

Figure 1. Test stall used in the two-choice test of sound localization. (A horse initiated trials by touching the observing plate with its nose and then touched the left or the right response plate to indicate whether a sound had been emitted from a loudspeaker to the left or right of midline. Correct responses were rewarded by delivering water into the bowl. The hardware-cloth panels separating the response plates served to prevent an animal from responding too rapidly. From "Sound Localization in Large Mammals: Localization of Complex Sounds by Horses" by H. E. Heffner and R. S. Heffner, 1984, *Behavioral Neuroscience, 98,* p. 543. Copyright 1984 by the American Psychological Association. Reproduced by permission.)

& Heffner, 1983; H. Heffner & Heffner, 1984). They were housed in outdoor pens and maintained on a standard diet of mixed grain and hay. Water, used as a reward, was available only during test sessions, and the animals were weighed daily to monitor their deprivational state.

At the beginning of testing, each animal was sedated with xylazine (0.8 mg/kg), and each external auditory meatus was carefully inspected. The ears of each animal were found to be free of obstruction or disease. In addition, behavioral audiograms of the same animals gave no indication of abnormality (R. Heffner & Heffner, 1983).

Behavioral apparatus. Testing was conducted on the grounds of the Kansas State Agriculture Experiment Station in Mound Valley, Kansas. The building in which the tests were conducted was relatively isolated from the rest of the station and its ventilating system was turned off during testing. These features, combined with the rural location, provided a quiet environment for auditory testing.

In order to attenuate outside noise and to reduce sound reflection, testing was conducted in a large room (7.2 × 5.5 × 2.4 m), which had the walls and ceiling lined with fiberglass and sound-absorbing panels (Cellotex). A room adjacent to the test room was used to house the test equipment and to observe the horses on closed-circuit television.

During testing, the animal was confined to a rectangular stall (2.4 × 1 × 1.5 m) mounted on a sawdust-covered wooden floor (Figure 1). Three metal response plates and a water bowl were located at the front of the stall within easy reach of the horse. The water bowl, located below the response plates, was connected with tubing to a 50-liter water reservoir. An electrically operated water valve inserted in the water line controlled the flow of water to the bowl, and the click that it emitted when it was operated served as a signal that water was being delivered. Each of the response plates was connected to a separate sensing switch which detected when the animal made contact with it.

Acoustical apparatus. Tones were produced by an oscillator (Hewlett-Packard 200CD), then led through an attenuator (Hewlett-Packard 350D), a rise–decay gate (Coulbourn S84-04), a programmable attenuator (Coulbourn S85-08), a band-pass filter (Krohn-Hite 3202), an amplifier (Coulbourn S82-24), and finally to one of two 3-in. (7.6-cm) woofers for frequencies between 125 Hz and 8 kHz or to one of two 1¼-in. (3.1-cm) dome tweeters for frequencies of 16–25 kHz.

The speakers were mounted at ear level 30° to the left and right of midline (60° total separation) on a perimeter bar (1.5-m radius) which was centered on the animal's ears. The sound pressure levels of the tones were equated and measured at the position occupied by the animal's ears by a sound level meter (Brüel & Kjaer 2203 or 2608 microphone amplifier, 4131 or 4135 microphone, and 1613 filter). The tones used ranged in octave intervals from 250 Hz to 16 kHz, with 25 kHz also being used. The tone pips were 100 ms in duration with rise–decay times of 50 ms to avoid onset and offset transients. All tones were presented at an average intensity of 50 dB above threshold and were randomly attenuated through a 4-dB range to reduce the possibility that the animals might detect slight differences between speakers.

For comparison, a 100-ms burst of 8-kHz narrow band noise (50 ms rise–decay) was also used. It was produced by a noise generator (Lehigh Valley 1524) with the band-pass filter centered at 8 kHz (24 dB per octave roll-off). This stimulus was approximately 40 dB above the animals' thresholds.

Psychophysical procedure. A water-deprived animal was trained to initiate a trial by placing its nose on the center plate. This "observing response" served to center the animal's head and to trigger the presentation of a single tone pip from a loudspeaker to the left or right of the animal's midline. The animal was then rewarded with 35 ml of water if it touched the response plate on the same side as the active speaker. Touching the opposite response plate was followed by a short time-out of 3–15 s (signaled by dimming the lights in the test room) before a new trial could begin. A typical session lasted 1 hr during which an animal received 200–500 trials and consumed 6–17 liters of water.

The sequence of left and right trials was determined by a quasi-random schedule (Gellermann, 1933). Side preferences were avoided by using a correction procedure in which the correct side was not changed following an error. These nonrandom correction trials were not used in the computation of performance. The results to be reported here are for asymptotic performance for single tone pips at 60° separation.

Results and Discussion

The ability of the horses to localize single tone pips at a 60° angle of separation is shown in Figure 2. All 3 horses were able to localize the lower frequencies of 250 Hz, 500 Hz, and 1 kHz, though individual performance levels varied. Horse A performed at relatively high levels, but both Horse B and Horse C were unable to attain similar levels. However, it is interesting to note that in each case, performance improved as frequency was decreased. In other words, the lower the frequency, the better the animals were able to localize the tone.

In contrast, none of the animals were ever able to localize the higher frequency tones. As shown in Figure 2, all 3 horses performed at chance levels for tones of 2 kHz to 25 kHz. Indeed, in spite of training efforts involving thousands of trials, the animals never showed any sign of being able to localize high frequencies even when the angle of separation was increased to angles ranging from 90° to 180°.

In order to ensure that there was nothing unusual about the sound field, three human observers were asked to localize tones in the horse apparatus with the same stimuli and

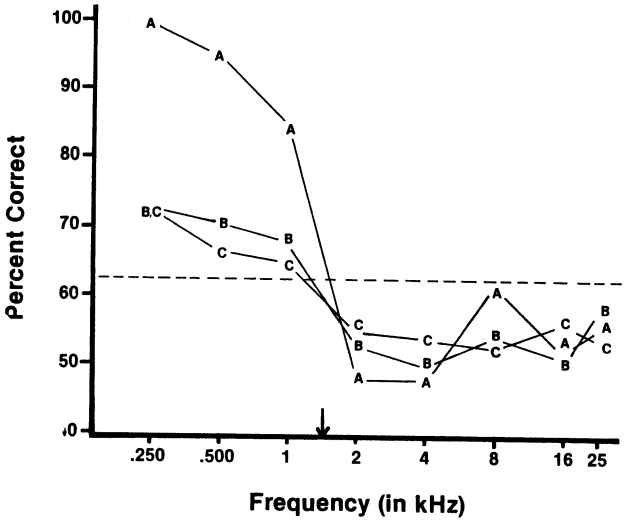

Figure 2. Sound-localization performance on the two-choice task for single, 100-ms pure-tone pips located 30° to the left and right of midline. (A, B, and C represent the 3 horses, and the dashed line indicates chance performance. Arrow indicates frequency above which the phase cue is ambiguous).

procedure. With their heads in the same position as that occupied by the horses during the observing response, each observer was able to perfectly localize all of the tones from 250 Hz to 16 kHz at a separation of 60°. Thus, there was no indication that any aspect of the apparatus or environment might have prevented the horses from localizing high frequencies.

The performance of the horses on this task may be interpreted in terms of their ability to make use of two binaural localization cues, time and intensity. According to the duplex theory of sound localization, low-frequency tones are localized through use of the phase cue ($\Delta\phi$), a subset of the class of time cues (Δt; for a review, see Woodworth & Schlosberg, 1965). Thus, the perceived azimuth of a low-frequency sound source depends on the difference in the phase of the sound reaching the two ears. But as frequency is increased, the phase cue becomes ambiguous (when the difference between the distances of the two ears from the sound source equals one half of the wave length of the tone). Above this point of phase ambiguity, higher frequencies can be localized accurately only through use of the intensity-difference cue (ΔI). The ΔI cue, however, is of much less use for low frequencies which bend around the head with little or no attenuation.

At a 60° loudspeaker separation (each speaker 30° from midline), the calculated frequency of ambiguity for the phase cue for these horses is 1500 Hz (indicated by the arrow in Figure 2; see Kuhn, 1977, or Brown, Beecher, Moody, & Stebbins, 1978, for the formula for calculating the frequency of phase ambiguity); that is, the phase cue should be available for all frequencies below 1500 Hz. However, it is unlikely that usable intensity differences exist for these frequencies, as empirical measurements have indicated that intensity differences for horses below 2 kHz are either small or unreliable (cf. H. Heffner & Heffner, 1984). Thus, the ability of the horses to localize 250 Hz, 500 Hz, and 1 kHz indicates that they are able to use the binaural phase-difference cue to localize sound.

Because the phase cue was available in this test only up to 1500 Hz (for the 60° speaker separation), localization of higher frequency tones would depend on the use of the intensity-difference cue. Yet in spite of the fact that these tones were within the range of frequencies that produce reliable intensity differences (H. Heffner & Heffner, 1984), the horses were never able to localize them. Thus, the complete inability of the horses to localize tones from 2 kHz to 25 kHz indicates that horses lack the ability to use the intensity-difference cue.

The inability to localize high-frequency pure tones, however, does not mean that horses are incapable of localizing high-frequency sounds in general. When presented with a burst of 8-kHz band-pass noise (60° separation), all of the animals were able to successfully discriminate the locus of the sound (with Horses A, B, and C performing at 95%, 80%, and 70% correct, respectively). However, the noise burst contained a sound-localization cue in addition to binaural intensity differences. Specifically, it has been demonstrated that the auditory system can utilize interaural time differences to localize high-frequency sounds that have complex waveforms (e.g., McFadden & Pasanen, 1976). This is evidently accomplished by analyzing the interaural time difference present in the fluctuating envelope of the signal, in this case, the noise band. The pure tones used here, however, had no fluctuating envelope, and, presumably, only the intensity-difference cue was available for localizing high-frequency pure tones—a cue that the horses could not use.

In summary, the ability of the horses to localize pure tones below the frequency of ambiguity for phase cues and their inability to localize tones above the frequency of ambiguity indicate that horses can use binaural phase differences but not binaural intensity differences to localize sound. The ability of horses to localize high-frequency noise demonstrates that they can use the time cues available in the envelope of the signal.

Experiment 2: Conditioned Avoidance Tone Localization

Though the results of the two-choice tests just described suggest that horses are unable to use the binaural ΔI cue to localize sound, we were reluctant to accept this conclusion without verification from an entirely different procedure. Accordingly, further tests of the ability of horses to use the binaural Δt and ΔI cues were undertaken with different animals in a different setting and with a shock avoidance procedure.

Shock avoidance was chosen for this test because it requires little learning or motor skill on the part of the animal. To perform well, an animal merely drinks water from a bowl then momentarily withdraws when a warning sound signals impending shock. This type of task has been used successfully to assess hearing in brain-damaged or otherwise intractable animals (e.g., Masterton, Heffner, & Ravizza, 1969; Ravizza & Masterton, 1972).

Method

Subjects. Two new 3-year-old horses (Shetland ponies), Horse I (100-kg female) and Horse J (104-kg male), were acquired for this test. The animals had also been used in previous tests of sound localization (H. Heffner & Heffner, 1984). They were housed in indoor pens and maintained on mixed grain and hay. Water was used as the reward and was available only during testing. Their ears were inspected and found to be free of any signs of obstruction or disease.

Behavioral apparatus. Details of the behavioral apparatus have been described elsewhere (H. Heffner & Heffner, 1984). Briefly, testing was conducted in a laboratory room (3.2 × 2.5 × 2.4 m) with the walls lined with acoustic foam, the floor covered with carpeting, and burlap hung loosely from the ceiling. A nearby room was used to house the test equipment, and the animals were observed on closed-circuit television.

During testing, a horse was confined to a rectangular stall (145 × 60 × 80 cm) specially constructed so as to reduce possible interference with the sound field around the animal's head (Figure 3). A water bowl was located in a position that allowed the horse to drink comfortably while centering its head in front of a perimeter bar from which loudspeakers were suspended. The water bowl was connected by plastic tubing to a solenoid-operated water valve and water reservoir located outside the test room. A touch-sensing switch, connected at one end to the water bowl and at the other end to the flank of an animal with an electroencephalographic electrode, served to detect when the animal made contact with the bowl. Mild electric shock

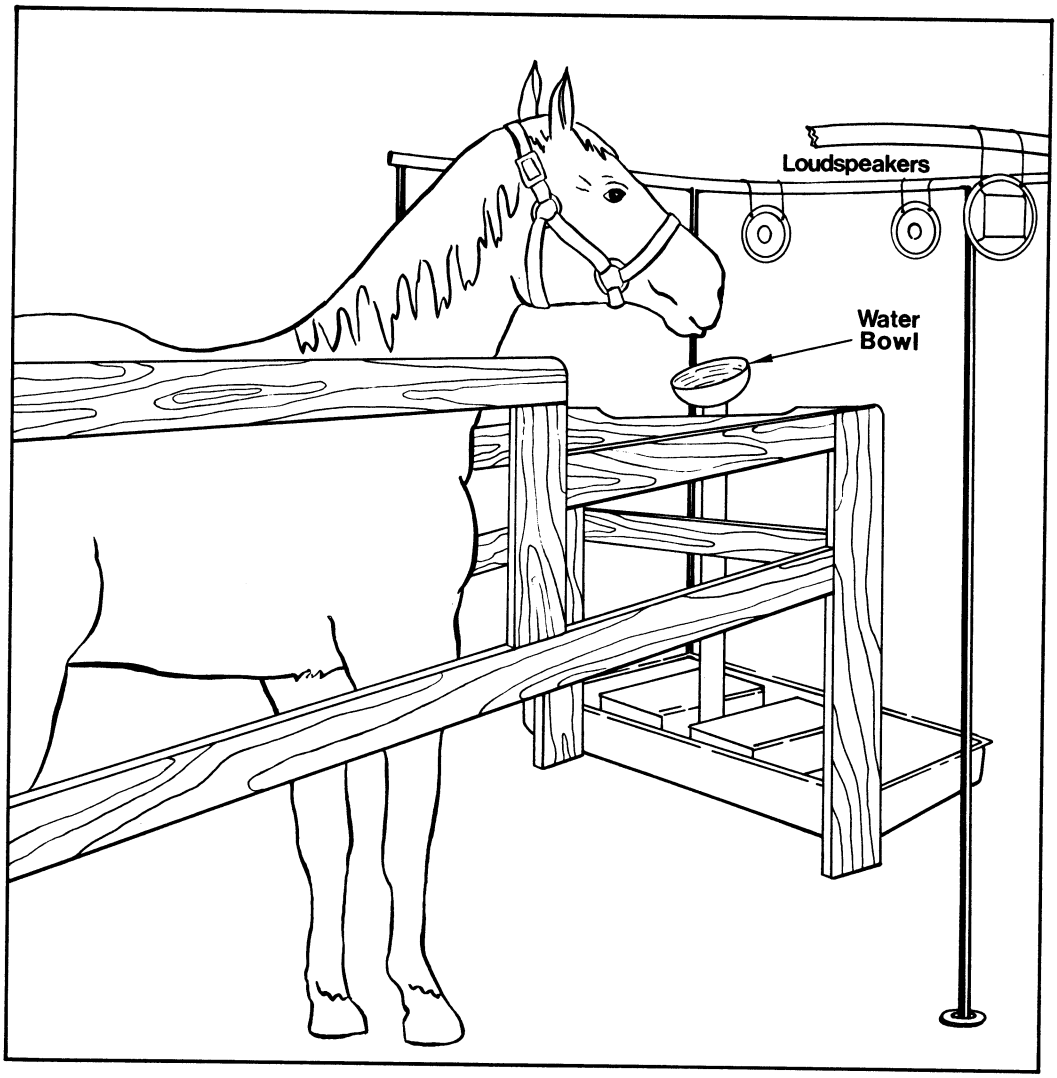

Figure 3. Test stall used in the conditioned avoidance test of localization. (An animal was trained to maintain steady contact with the water bowl in order to obtain a trickle of water. It was then trained to cease drinking when a sound was emitted from a loudspeaker to its left side in order to avoid a mild electric shock. Sounds emitted from speakers to the right were not followed by shock, and the animals soon learned to continue drinking when they were presented. From "Sound Localization in Large Mammals: Localization of Complex Sounds by Horses" by H. E. Heffner and R. S. Heffner, 1984, *Behavioral Neuroscience, 98,* p. 546. Copyright 1984 by the American Psychological Association. Reproduced by permission.)

was provided by a shock generator connected to the water bowl and flank electrode.

Acoustical apparatus. Pure-tone pips and an 8-kHz high-pass noise band were generated and presented in the same manner as and with the same equipment used in Experiment 1. All test stimuli were 100 ms in duration with a 50-ms rise–decay time.

Psychophysical procedure. The conditioned avoidance procedure used here is the same as that described elsewhere (H. Heffner & Heffner, 1984). A thirsty animal was trained to make steady contact with the water bowl with its mouth in order to receive a steady trickle of water (approximately 140 ml/min). The animals were trained to maintain contact with the water bowl while a tone pip was presented once every 5 s from a speaker to the right of midline. They were then trained to break contact with the bowl whenever a tone pip was emitted from a speaker to the left of midline in order to avoid a mild electric shock which was delivered through the water bowl 3 s after the onset of the tone. In order to provide feedback for successful avoidance, the lights in the test room were momentarily turned off each time shock was delivered. The lights thereby served to indicate that a warning trial was over and that the animal could return to drinking. Thus, cessation of contact with the water bowl was used as an indication that the animal had perceived a sound coming from the left side.

The sessions were divided into 3-s trials which were presented every 5 s (with a 2-s intertrial interval). A trial began with the presentation of a single tone pip which was emitted from a loudspeaker located on the animal's right (a safe or S trial) or left (a warning or W trial) side. The presentation of safe and warning trials was randomized,

with a warning trial likely to occur anywhere from 1 to 10 trials after the previous warning trial. No trial was given in the 5 s immediately following a warning trial in order to allow an animal sufficient time to return to the water bowl.

For the purpose of quantifying an animal's response, the duration of bowl contact was measured in 0.1-s increments beginning 2 s after the stimulus onset until 1 s later— at the end of the trial. This measured "time in contact" was then averaged separately for the right (S) trials and the left (W) trials for each frequency. A measure of discrimination could then be expressed in the form of a ratio $(S - W)/S$. In trained animals this measure varies from near zero (failure to discriminate) to one (perfect discrimination). In order to reduce the effects of spurious pauses, the results of a trial were automatically discarded if the animal was not in contact with the bowl at any time during the 1 s immediately preceding the trial, though the trial and shock were presented as usual. This criterion was applied equally to safe and warning trials and thus did not bias the results.

The animals were tested for their ability to discriminate the source of tone pips separated by 60° (i.e., 30° left and 30° right of midline). The frequencies of the tones ranged from 250 Hz to 16 kHz in octave steps. The tones were presented at an average of 50 dB above threshold and were randomly varied by ± 2 dB. Testing continued while the shock intensity and the animal's deprivation level were varied. Testing was considered complete for a frequency when asymptotic performance was reached, and the scores reported here represent the average of each animal's best three sessions. For comparison, the animals were also tested with an 8-kHz high-pass noise stimulus. Sessions lasted approximately 1 hr during which an animal received approximately 300 trials (approximately 18% of which were warning trials) and drank 6–9 liters of water.

Results and Discussion

Conditioned avoidance proved to be an easy task for these animals (cf. H. Heffner & Heffner, 1984). At the beginning of the tone-localization tests reported here, both horses were well-trained observers, having had approximately 130 sessions of sound-localization testing with clicks and white noise.

Figure 4 illustrates the ability of Horses I and J to discriminate the sources of pure tones separated by 60°. At low frequencies the animals had no difficulty performing the discrimination, results illustrating that the task itself was a simple one for them to perform. However, their performances declined markedly at 1 kHz and fell to chance for frequencies of 2 kHz and above (Mann-Whitney U, $p < .01$).

Comparison of two-choice and conditioned avoidance results. Despite the use of different animals in a different setting with an entirely different procedure, the results of this experiment are virtually identical to those of Experiment 1. In both experiments, the horses were unable to localize pure tones of 2 kHz and higher. It appears, then, that this result is not an artifact of a particular behavioral procedure. Instead, the inability of horses to localize pure tones in the frequency range in which the phase cue is ambiguous demonstrates that they lack completely the ability to use binaural intensity-difference cues.

Experiment 3: Unconditioned Orientation to Tones

A final test of the ability of horses to localize tones was conducted by using an *un*conditioned response. In this test, the orientation of 4 additional horses was recorded for 500-Hz and 8-kHz tone pips. The purpose of this test was to determine the unconditioned orientation response of horses to these tones as well as to extend our observations to additional animals.

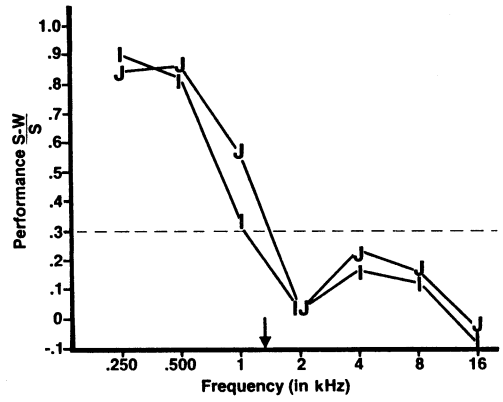

Figure 4. Sound-localization performance on the conditioned avoidance task for single 100-ms pure-tone pips. (I and J represent the 2 animals tested with this method. Dashed line indicates chance performance. Arrow indicates frequency above which the phase cue is ambiguous.)

Method

Subjects. Four naive horses were obtained for this experiment: Horse D, an 11-year-old Shetland gelding; Horse E, a 13-year-old quarterhorse gelding; Horse F, a 19-year-old paint mare; and Horse G, a 4-year-old Appaloosa mare. The animals were maintained on free feed and water. All were accustomed to human handling and entered the test stall with only moderate reluctance.

Behavioral apparatus. The test room and audio equipment were the same as in Experiment 1. Two loudspeakers were used and were placed 90° to the left and right of the animal's midline. To record the responses of the horses, a videotape recorder was connected to the television camera used to observe the horses. In addition, two light-emitting diodes were placed on the left and right sides of the stall out of sight of the horse but in view of the television camera. These were used to indicate on the videotape the onset of a tone as well as the side of the active speaker.

Behavioral procedure. An animal was led into the test stall, loosely tethered, and given a small amount of grain to eat. It was then allowed to remain undisturbed until it had adjusted to the unfamiliar surroundings (usually 15–60 min). The animal was then presented with a single tone pip (100 ms in duration, 50-ms rise–decay) of either 500 Hz or 8 kHz (50 dB above threshold) from one of the loudspeakers, and its head and pinna orientation movements were observed and recorded on videotape. Trials were initiated only when the animal was facing the midline, with its ears at the approximate level of the speakers. Trials were spaced 7–20 min apart with five trials being given per day. Five presentations of each frequency were made to each subject. The sequence of left-right and high-low frequency was determined by a quasi-random schedule (Gellermann, 1933).

Following testing, the videotapes were analyzed frame by frame to determine the direction and latency of any orienting response. Any movement of either the head or pinnae in a lateral direction within 1 s of stimulus onset was counted as an orientation response, though most of the orientations occurred within 350 ms of tone onset. The

Table 1
Unconditioned Orientation Response of Four Horses to 500-Hz and 8-kHz Tone Pips

Horse	500 Hz			8 kHz		
	Toward sound	Away from sound	Alerting response only	Toward sound	Away from sound	Alerting response only
D	5	0	0	1	1	3
E	5	0	0	2	2	1
F	4	0	1	1	3	1
G	5	0	0	1	1	3

Note. Each tone was presented five times to each animal.

responses to the tone pips were scored in one of three ways, as toward the source of the sound, away from the source of the sound, or an alerting response only with no lateral component occurring within 1 s following stimulus presentation.

Results and Discussion

An orientation response to a sound consisted of an alerting response in which the horse raised its head, followed by movement of the head or pinnae to one side or the other. As shown in Table 1, an orientation response toward the source of the sound occurred in almost every presentation of the 500-Hz tone ($p < .001$, multinomial distribution). The single exception was one trial in the case of Horse F in which the animal showed an alerting response without any noticeable turning of the head or pinnae. In contrast, no such consistent behavior was observed with the 8-kHz tone ($p > .05$). Although the horses occasionally oriented in the direction of the sound source, they were just as likely to orient in the opposite direction or to show only an alerting response without detectable orientation. Thus, the 4 horses demonstrated an observable orientation toward the source of a low-frequency tone but failed to show such a response to the source of a high-frequency tone.

The unconditioned responses of horses to tonal stimuli are similar to their trained responses and support the conclusion of the previous two experiments: Horses can make a directional response to the source of a sound when binaural phase differences are present, but not when binaural intensity differences provide the only cue. In addition, these results provide some insight into the response of horses to sounds in a more natural situation. As demonstrated here, a horse will correctly orient to a novel sound that contains time cues, whereas sounds that lack these cues will elicit either a random orientation response or merely a nondirectional alerting response.

Experiment 4: Description of the Superior Olivary Complex

The superior olivary complex (SOC) is the first nucleus in the mammalian brain stem where input from the two ears interacts and where disparities between the two signals can be detected. Indeed, it has been demonstrated in the cat and dog that cells in the medial superior olive (MSO) are sensitive to small binaural time differences in the range used for sound localization (Caird & Klinke, 1983; Galambos, Schwartzkopff, & Rupert, 1959; Goldberg & Brown, 1969) and cells in the lateral superior olive (LSO) are sensitive to small binaural intensity differences (Boudreau & Tsuchitani, 1968; Tsuchitani & Boudreau, 1966), though neither nucleus contains such units exclusively. It has also been observed that the size and configuration of the SOC vary greatly among mammalian species (e.g., Irving & Harrison, 1967; Moore & Moore, 1971) and at least some of this variation has been linked to differences among certain species (i.e., hedgehog, rat, tree shrew, cat) in the ability to use the binaural cues for localizing sound (Masterton, et al., 1975). Thus it was of interest to examine the SOC of the horse to determine whether the horse's inability to use the ΔI cue is associated with any unusual aspects of its SOC, in particular the lateral nucleus.

Method

The materials used consisted of the normal brains of 2 horses, a 3-year-old and a 20-year-old. The animals were euthanized and then perfused through the carotids with 0.9% NaCl followed by 10% formalin. The brain stems were subsequently prepared for frozen sectioning by immersion in a graded series of glycerine solutions ending with 25% glycerin. One brain stem was cut in the coronal plane with section thicknesses of 20 μm, 40 μm, and 40 μm, and the sequence was repeated throughout the brain stem. The other brain stem was split along the sagittal plane, with one half being cut coronally and the other half horizontally. Both halves were cut 25, 25, and 50 μm (and the sequence was repeated). Alternate series of sections of both thick and thin sets in both planes were stained with thionine and protargol stains.

The brain stems from the horses were then compared with similarly stained brain stems of numerous other species. Cats and rats were chosen for illustration because they are common mammalian models for studies of hearing and the central auditory system. Both species are capable of using both Δt and ΔI (Masterton et al., 1975), with the cat being a more accurate localizer than the rat (cf. Casseday & Neff, 1973; H. Heffner & Heffner, 1985; Kelly, 1980).

Results and Discussion

Because the morphology of the mammalian superior olivary complex varies greatly between species, the establishment of homologies between nuclei can be difficult. Although position relative to other nuclei and fiber bundles is often helpful, it can be misleading, particularly when some components are missing or displaced by enlarged structures nearby. An analysis based on internal morphological features (i.e., cell structure and orientation, synaptic endings, neuropil) avoids these difficulties but may not be entirely foolproof for species that are very deviant. Ideally, homologies would be based on anatomical connections and electrophysiological response properties of the neurons themselves. Because these latter data are not available for the horse (or for most other mammals), we have relied on location, gross morphology, and microscopic appearance to make a tenative identification of the superior olivary complex in the horse.

Configuration. Figure 5 illustrates the configuration of the SOC for the horse, cat, and rat. The section drawn for each species shows the LSO at its greatest extent. Two observations can be made on the basis of this illustration: First, the horse

possesses three cell groups in the typical location of the SOC in mammals, and, second, the configuration of the nuclei is unlike that of either the cat or the rat. The nucleus labeled MSO in the drawing of the horse is broad mediolaterally and appears very different from the dorsoventrally oriented cell column of the MSO in cats and rats. The nucleus labeled LSO is smaller in relation to the size of the brain stem than it is in either cats or rats. It also lacks the very dense neuropil and convolutions characteristic of the LSO in these and many other species. The nucleus labeled MNTB (medial nucleus of the trapezoid body), which relays input from the contralateral cochlear nucleus to the LSO, is correspondingly small in the horse.

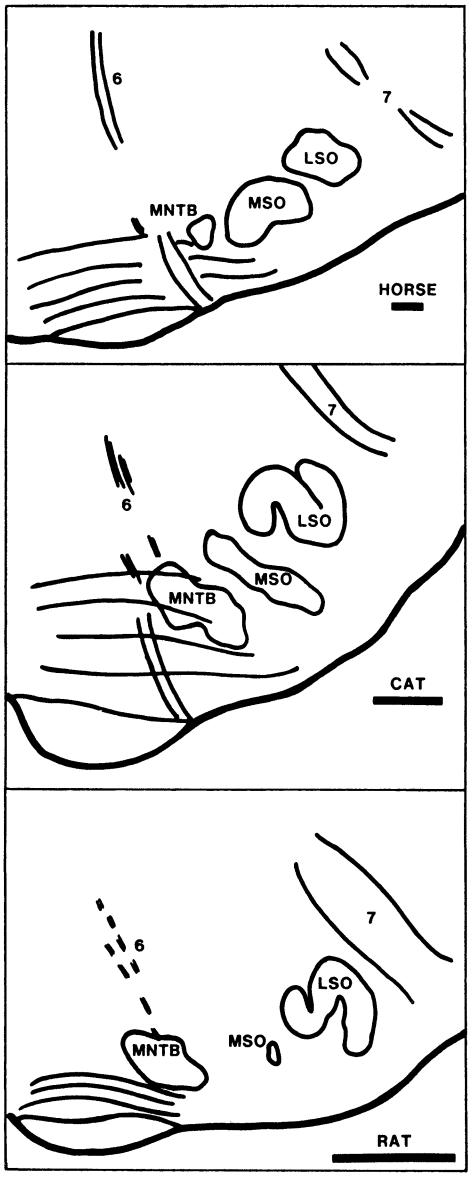

Figure 5. Tracings of the right superior olivary complex of a horse, cat, and rat. (For facilitation of comparison, sections are enlarged so that the region of the SOC, i.e., distance between the sixth and seventh nerves, is approximately equal for each species. LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; 6, abducens nerve; 7, facial nerve. Scale bars equal 1 mm.)

Microscopic appearance. Figure 6 shows a photomicrograph of the SOC of a horse. The large cell group labeled MSO contains multipolar and bipolar neurons. Unlike the arrangement of the MSO in most species, however, there is not a densely packed vertical column of bipolar cells with their primary dendrites oriented perpendicularly to the borders of the nucleus. Instead, the cells are loosely scattered and lack the columnar arrangement characteristic of this nucleus. The fibers from the trapezoid body appear to enter the MSO primarily from below rather than from each side as in other species. Thus, the nucleus labeled MSO in the horse does not fulfill the usual criteria for qualifying as an MSO (cf. Irving & Harrison, 1967). Indeed its only qualifications for being so labeled are its location in the brain stem and its slightly denser neuropil which is revealed by the protargol stain.

The prominent bipolar neurons of the MSO of most species (that are so sparse in the horse) are the cells generally considered to be the anatomical substrate of the binaurally driven units (Galambos et al., 1959; Goldberg & Brown, 1969). However, in addition to these, both multipolar cells and rostocaudally oriented bipolar cells are present in the MSO of the cat (Scheibel & Scheibel, 1974; Schwartz, 1977) and may be the main remaining cell type in the horse. These cells have not yet been associated with a particular response pattern or function.

Because the nucleus labeled MSO in the horse is so atypical in appearance, electrophysiological studies of its cells' response patterns and anatomical tracing of their connections will be important in order to determine whether the nucleus labeled MSO in Figures 5 and 6 is actually the MSO and not an enlarged periolivary nucleus.

The cell group labeled LSO is also unusual in its microscopic appearance. Again, though the protargol stain shows it to be enmeshed in neuropil denser than the surrounding areas, and though it is found in the typical location in the brain

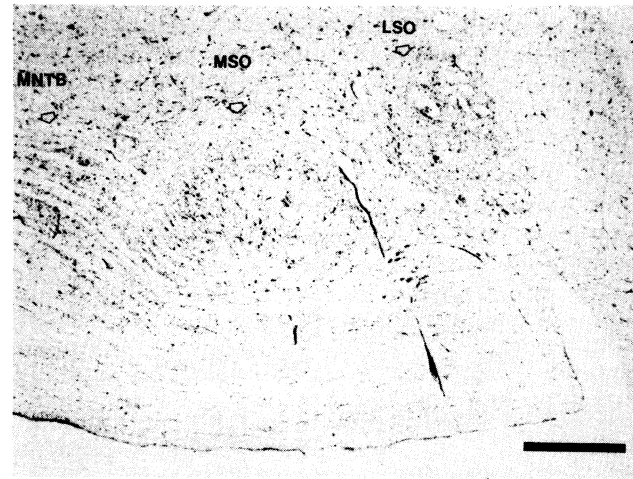

Figure 6. Photomicrograph of a thionine-stained coronal section through the superior olivary complex of a horse. (Scale bar equals 1 mm. See caption to Figure 5 for abbreviations. See Plate F.)

stem, its cells are loosely arranged and apparently few in number. There is no characteristic dense laminar arrangement of fusiform cells oriented with their long axis radial to the contours of the nucleus. Nor was there evidence in the horizontal sections of longitudinal sheets of neuropil and longitudinally oriented neurons as described for the cat (Scheibel & Scheibel, 1974).

Because the morphology of the cells in the MSO and LSO is intimately related to their function, the morphological differences in these nuclei in the horse are of potential significance in interpreting their function; for example, the bipolar neurons of the LSO should receive input from the ipsilateral cochlear nucleus on one set of dendrites and input from the contralateral cochlear nucleus (via the nucleus of the trapezoid body) on the other set (e.g., Tsuchitani, 1978). The characteristic appearance of the nucleus is due to the flattened appearance of the cells and the dense fibers approaching them from either side (for a recent review, see Aitkin, Irvine, & Webster, 1984). When this arrangement of neurons and neuropil is altered, it suggests that their function may also be altered. Thus, the differences in the configuration and microscopic appearance of the horse SOC from what is seen in more familiar species suggest the possibility of functional differences in the ability to localize sound.

General Discussion

The results of these experiments have demonstrated the inability of horses to localize high-frequency tones in a variety of situations. Altogether, three different procedures were used on a total of 9 animals, with the tests being conducted in two different test chambers. Yet, in spite of the fact that humans could easily localize at all frequencies in the same apparatus, none of the horses showed any sign of being able to localize pure tones of 2 kHz or higher. Thus, it appears that the inability of horses to localize high frequencies is a genuine phenomenon and is not the consequence of using a particular behavioral procedure, acoustic environment, or individual animal.

The inability of the horses to localize high-frequency tones demonstrates that they are unable to use binaural intensity differences, or ΔI, to localize sound. As described in Results and Discussion of Experiment 1, of the two binaural cues for localizing pure tones, only ΔI would be available for horses for frequencies above 1500 Hz at a loudspeaker separation of 60°. The ability of the horses to localize tones of 1 kHz or lower and narrow bands of high-frequency noise (with the consequent envelope) demonstrates that they can use time cues. Thus, horses can use binaural time differences, but not binaural intensity differences, to localize sound.

Horses Compared With Other Mammals

Most mammals studied so far appear able to use both binaural time- and intensity-difference cues. These include the tree shrew, macaques, squirrel monkey, humans, white rat, kangaroo rat, cat, and elephant (Brown et al., 1978; Casseday & Neff, 1973; Don & Starr, 1972; H. Heffner & Masterton, 1980; R. Heffner & Heffner, 1982; Masterton et al., 1975; Mills, 1972). However, although the horse is the first mammal known to completely lack the ability to use ΔI, it has been known for some time that the hedgehog lacks the ability to use $\Delta\phi$, and possibly Δt itself (Masterton et al., 1975). Furthermore, though the Indian elephant can use both cues, it does not appear to use ΔI as accurately as $\Delta\phi$ and has great difficulty in localizing tones in the upper octaves of its hearing range. It seems, then, that the degree to which mammals use both binaural cues may vary over a wide range from animals that rely equally on both cues to those that can use only one of the binaural cues.

Given the importance of sound localization for survival, the question arises as to why species would fail to take advantage of both time and intensity cues. Though it might be argued that in such a case, one of the cues was not physically available, this does not seem to be true in the case of horses. In spite of the fact that a horse's ears are near the top if its head, it has been demonstrated that even the head of a small pony is sufficient to generate large intensity-difference cues at high frequencies (H. Heffner & Heffner, 1984). Likewise, the functional interaural distance of the hedgehog is sufficiently large to generate usable time cues, as the hedgehog is nearly twice the size of the kangaroo rat and laboratory rat, animals that have been shown to easily use $\Delta\phi$ (H. Heffner & Masterton, 1980; Masterton et al., 1975). Therefore, the failure of the horse and hedgehog to use one of the binaural difference cues is not due to the physical absence of those cues.

Neuroanatomical Correlates of Sound Localization

The horse possesses nuclei that may correspond to the MSO and LSO, though both nuclei are so atypical in their gross configuration and in their microscopic appearance as to suggest that their function in binaural hearing may differ from that in other mammals. Unlike the hedgehog, which has no detectable MSO and lacks the ability to use $\Delta\phi$, the horse has a nucleus that appears to be a remnant of an LSO, even though the horse lacks the ability to use ΔI. It may be that the LSO of the horse has lost the high-frequency binaurally activated cells prominent in other species and instead consists only of low-frequency monaurally responsive units like those found in the lateral limb of the LSO of the cat (Guinan, Norris, & Guinan, 1972).

The possible presence of a small LSO in the horse, accompanied by an inability to analyze binaural intensity disparities, must be contrasted to the case of humans in which an even smaller LSO (Strominger & Hurwitz, 1976) is accompanied by acute ΔI discrimination (Mills, 1972). Differences such as these suggest that the physiological functions of the LSO that have been described for the cat, a species in which the nucleus is quite prominent, may not be the same in other mammals. Because there seem to be important differences between species, it would be of interest to determine both the anatomical connections and response properties of neurons in the LSO of the horse. Such information on a species that differs so greatly from the cat in both anatomy and behavior could provide insight to the nature of the contribution of the SOC to audition.

Sound Localization and High-Frequency Hearing

It has previously been proposed that the need to use binaural frequency–intensity spectral differences (Δfi) to localize sound has provided the main source of selective pressure for the evolution of high-frequency hearing in mammals (Masterton & Diamond, 1973; Masterton et al., 1969). This proposition is based on the observation that high-frequency hearing is directly correlated with the functional distance between the two ears. Mammals with small heads, and therefore close-set ears, are known to be better able to hear high-frequency sounds than species with large heads and wide-set ears. More precisely, high-frequency hearing varies inversely with the functional distance between the ears (for a recent review, see R. Heffner & Heffner, 1983).

The relation of Δfi to high-frequency hearing has been ascribed to the reliance of mammals on the use of binaural Δfi to localize sound. Briefly, both of the two binaural cues for sound localization, Δt and Δfi, depend on the functional distance between the two ears and the sound shadow of the head and pinnae; that is, the farther apart the ears, the larger will be the two cues for any given direction of a sound source. Whereas both of these cues are readily available to animals with large heads, the effectiveness of the Δt cue is diminished in animals with functionally close-set ears; that is, the available time difference may be so small that the nervous system can detect only gross changes in sound direction. However, an animal with a small head always has a Δfi cue available, providing only that it is able to perceive frequencies that are high enough to be shadowed by its head and pinnae. Therefore, given the ecological importance of sound localization, animals with functionally close-set ears are subjected to more selective pressure to hear high frequencies than are animals with more widely set ears.

The key to this explanation of mammalian high-frequency hearing, then, is that mammals need to hear high frequencies in order to make use of the binaural Δfi cue. However, binaural ΔI is a simplified form of Δfi, and, given the inability of horses to use ΔI, the conclusion is that they cannot use Δfi. Because the 33-kHz high-frequency hearing limit of the horse is significantly higher than that of either humans or elephants, both of which use Δfi, it appears that the high-frequency hearing ability of the horse cannot be due to the need to use Δfi.

Though the horse does not use Δfi, there is reason to suspect that it nonetheless requires high-frequency hearing in order to localize sound. It has been well established that high frequencies are required for monaural localization involving pinna cues (e.g., Butler, 1975; Searle, Braida, Davis, & Colburn, 1976). Such cues are important to prevent front-back reversals, that is, in determining whether a sound is coming from in front or from behind. Indeed, preliminary evidence has shown that horses are unable to discriminate between front and rear sound sources unless they contain high frequencies (H. Heffner & Heffner, 1983). Thus, there is reason to believe that horses require high-frequency hearing in order to use monaural pinna cues to localize sound.

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Received December 20, 1984
Revision received April 10, 1985 ■