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ORIGINAL PAPER

Hearing and sound localization in Cottontail rabbits, *Sylvilagus floridanus*

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Abstract
Cottontail rabbits represent the first wild species of the order of lagomorphs whose hearing abilities have been determined. Cottontails, *Sylvilagus floridanus*, evolved in the New World, but have spread worldwide. Their hearing was tested behaviorally using a conditioned-avoidance procedure. At a level of 60 dB SPL, their hearing ranged from 300 Hz to 32 kHz, a span of 7.5 octaves. Mammalian low-frequency hearing is bimodally distributed and Cottontail rabbits fall into the group that hears below 400 Hz. However, their 300-Hz limit puts them near the gap that separates the two populations. The minimum audible angle of cottontails is 27.6°, making them less acute than most other species of mammals. Their large sound-localization threshold is consistent with the observation that mammals with broad fields of best vision require less acuity to direct their eyes to the sources of sound.

Keywords Behavioral audiogram · Low-frequency hearing · Comparative hearing · Sound localization and vision · Animal psychophysics

Introduction

The Order Lagomorpha, with approximately 90 species, includes rabbits, hares, and pikas (Melo-Ferreira and Alves 2018). Currently, the only available audiogram for this Order is that of the domesticated Old-World rabbit, *Oryctolagus cuniculus*, a burrowing species (Heffner and Masterton 1980). To extend our survey of mammalian hearing to include a non-domesticated species in this group, we report here the audiogram of the Eastern cottontail rabbit (*Sylvilagus floridanus*).

The Cottontail rabbit is a member of a New-world genus native to much of North and Central America, including the northern parts of South America (Chapman et al. 1980). It is a smaller non-burrowing species, nesting in slight depressions, that can be compared to the larger domesticated representatives of Old-world burrowing rabbits (e.g., New Zealand White and Dutch Belted) that have served as models for mammalian sound localization (e.g., Blanks et al. 2007). Both the audiogram and noise-localization thresholds of three Eastern cottontails were determined for comparison with those of other mammals.

Methods

The rabbits were tested using a conditioned-avoidance procedure in which a thirsty animal was trained to maintain mouth contact with a water spout to receive a steady trickle of water. Warning sounds were presented intermittently, followed at their offset by a mild electric shock delivered via the spout. The animals learned to avoid the shock by breaking contact with the spout when they heard a warning sound. The audiogram was determined for pure tones ranging from 125 Hz to 64 kHz. Sound-localization acuity (minimum audible angle) was determined for 100-ms broadband noise pulses centered left and right on the midline in the azimuthal plane.

Subjects

Three Eastern cottontail rabbits, S. floridanus (two females A, B, and one male C), were wild trapped in Lucas County, Ohio, and maintained in the laboratory. They were approximately 6 months old and weighed 918–1099 g on ad libitum feed at the beginning of testing. They were housed in stainless steel cages (61 × 46 × 70 cm) and given free access to rabbit chow supplemented by occasional fruits and vegetables.

While on test, the animals received their water only in the test sessions and were weighed daily to monitor their deprivational state. During testing, they maintained at least 80% of their ad libitum weights and returned to those weights within a few days of ad libitum food and water.

Behavioral apparatus

All testing was carried out in a double-walled chamber (IAC model 1204, 2.55 × 2.75 × 2.05 m). To reduce sound reflection, the floor was carpeted and the walls and ceiling were lined with egg crate foam. All acoustic and behavioral equipment was located adjacent to the chamber and the rabbits were observed over closed-circuit television.

The test cage measured 55 × 31 × 38 cm and was constructed of 1-in (2.54-cm) welded wire mesh. The legs supporting the test cage were placed on 8-cm-thick foam pads as a precaution against substrate-borne vibrations. In the front of the cage, a water spout protruded through the floor to a comfortable drinking height. The spout consisted of 15-gauge stainless steel tubing with a 1.5 × 2.5 cm stainless steel oval welded to the tip serving as a lick surface. The tip of the spout protruded 5 cm above the cage floor, below the level of the animals' ears, thus minimizing obstructions between the ears and the loudspeakers. The water spout was connected via plastic tubing to a 50 mL syringe pump (Thompson et al. 1990) located outside the test chamber. The pump supplied a slow trickle of water as long as an animal maintained contact with the spout. The water delivery rate was adjusted, so that the animals could obtain their daily water in a single test session lasting 35–65 min. Requiring the animals to keep their mouths on the water spout served to keep their heads in a fixed position relative to the loudspeakers. A contact circuit, connected between the spout and cage floor, detected when a rabbit made contact with the spout and activated the syringe pump. In addition, a shock generator was connected between the spout and the cage floor to provide feedback and a mild cost for failing to respond to warning sounds. The shock (0.3 s) was adjusted for each rabbit to the lowest level that elicited a reliable avoidance response. Finally, a 15-W light was mounted approximately 0.5 m below the cage and was turned on and off simultaneously with the shock to indicate to the animal when a shock had been delivered, and when it was safe to return to the spout at the end of successful detection trials.

Acoustical apparatus

Audiogram

Pure tones from 125 Hz to 64 kHz were produced using a signal generator (Krohn-Hite 2400 AM/FM) and were continuously verified by a frequency counter (Fluke 1900A). The signal was shaped by a rise/decay gate (Coulbourn S84-04) allowing 10 ms rise/decay times for all frequencies of 1 kHz and higher. Longer rise/decay times were used at lower frequencies to allow the signal to reach full voltage (and fall to zero voltage) over at least ten cycles. For the audiogram, pure tones were presented as four pulses of 400-ms duration with 100 ms between pulses. The intensity of the tones was adjusted in 5-dB steps using an attenuator (Hewlett Packard 350D), the linearity of which was calibrated throughout the voltage range used for the different intensities being tested. The electrical signal was then bandpass filtered (Krohn-Hite 3550; ± of the to(ont of).5esing an,]TJ .00

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signals indicated that any overtones were more than 10 dB below the animals’ thresholds.

Sound localization

To determine minimum audible angle, a single 100-ms broadband noise burst (2–45 kHz) was emitted from one of

For additional discussion of the method, see Heffner and Heffner (1995), Heffner et al. (2006), Koay et al. (1998).

Absolute threshold for tones was defined as the intensity at which the performance measure (Corrected Detection) equaled 0.50, usually obtained by interpolation. Chance performance is also noted and is defined as the score for

Discussion

mammals, this does not exclude the influence of other factors. Indeed, given a correlation between high-frequency hearing and functional interaural distance of $r = 0.76$, it appears that although functional head size accounts for about 58% of the variance in high-frequency hearing, 42% remains unaccounted for. Some possible factors include communication that relies on high frequencies in some species (e.g., Noirot and Pye 1969; Ehret 2003). Another potential factor is the high-frequency pinna cues that prevent front–back confusions (e.g., Butler 1986; Heffner et al. 1995). The pinnae also serve as directional filters that allow animals to from its tight connection to the skull to reduce noise transmitted to the ear via bone conduction (Mao et al. 2020). This involved changes in the jaw bones that led to some jaw bones evolving into the three-boned middle ear thereby breaking the direct connection between the jaw and the ear and reducing noise from chewing. The three-boned middle ear seems to have enabled more effective transduction of high frequencies that, in turn, provided the opportunity to take advantage of the directional filtering properties of the pinnae at high frequencies.

Virtually all modern mammals have pinnae, but they have only recently been documented in the fossil record of an early mammal, Spinolestes xenarthrosus, from about 125–127 million years ago (Ma) (Martin et al. 2015). Thus, pinnae were present before the rearrangement of the jaw bones to form the middle ear 123 Ma (Mao et al. 2020). We suggest that pinnae played a more important role than usually recognized in the early evolution of high-frequency hearing. If that is the case, some aspect of pinna size may prove to be a factor that accounts for some of the remaining variance in high-frequency hearing in mammals.

Low-frequency hearing

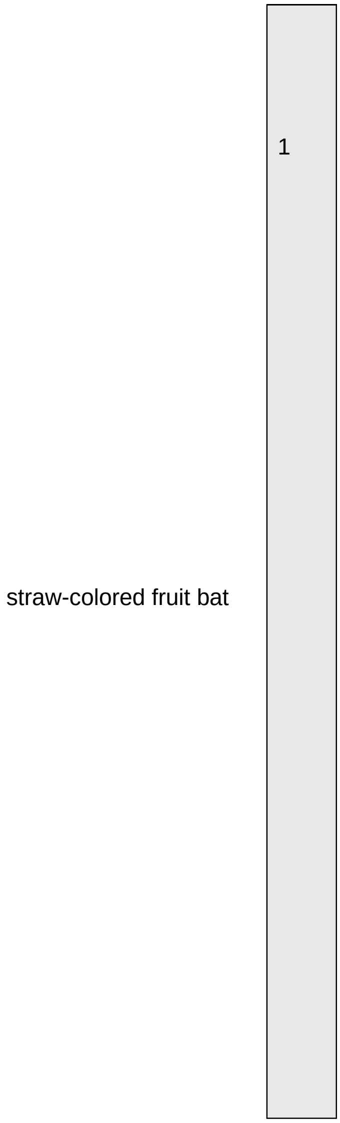
Low-frequency hearing limits in mammals encompass a range of 9.24 octaves, twice as broad as the 4.66-octave range of high-frequency hearing limits (5.9–150 kHz). More sur-

It is well known that some desert rodents (e.g., Gerbils and Kangaroo rats) and chinchillas have unusually large bullae thought to make their good low-frequency hearing possible. However, large bullae are not essential, because many rodents in the squirrel family, as well as least weasels, are equally small and have similar low-frequency hearing without enlarged bullae (Heffner and Heffner 1985b; Heffner et al. 2001). Such comparisons suggest that there must be more than one morphological adaptation to accommodate low-frequency hearing.

It seems unlikely that the length of the basilar membrane is a constraint on low-frequency hearing, because there are many species that hear both very low and high. For exampl Domestic cats at a level of 60 dB hear from 0.055 to 79 kH (Heffner and Heffner 1985a); Chipmunks hear 0.039–52 kHz (Heffner et al. 2001); Gerbils hear 0.036 Hz–58 kHz (Ryan 1976); Least weasels hear 0.050–60 kHz (Heffner and Heffner 1985b). Because many species that hear below abo 400 Hz also hear well at high frequencies, low-frequenc hearing is not reliably correlated with high-frequency hearing ($r = 0.154$, $p = 0.360$). However, among species that do not hear low frequencies well (species on the right side o Fig. 4), there is a moderate correlation ($r = 0.605$, $p = 0.0017$) accounting for about 36% of the variance in high-frequenc hearing within that group. Thus, it seems that a substantia portion of mammals that hear in air have been able to exte their low-frequency hearing below 400 Hz without comparable sacrifice of high-frequency sensitivity. It is as if some mammals have a second means of transducing sound th permits them to extend their hearing range below 400 H It has been proposed that such a mechanism might be t temporal code for frequency in the cochlea (Heffner et al 2001). There have been relatively few comparative studie of the actual frequencies over which the temporal and plac mechanisms operate to enlighten this question (e.g., Walk et al. 2011; for a review, see Vater and Kossl 2011; Hei and Peterson 2017; Verschooten et al. 2019); almost no of these comparative studies were carried out on speci with poor low-frequency hearing. Yet, evidence continues t accumulate, showing that cochlear mechanisms in the lowe frequency apical region are different from those in the rest o the cochlea, but that such discontinuities are hard to find i species that do not hear low frequencies (Greenwood 199 Sasmal and Grosh, 2019). We may eventually discover th mechanisms underlying the bimodal distribution of low-frequency sensitivity, but the selective pressures that led this difference remain unexplored.

Sound localization

Cottontail rabbits, with their mean minimum audible angle of 27.6°, are relatively poor localizers. Only Kangaroo rats, Gerbils, Domestic mice, Cattle, and the subterranean species have worse localization acuity (Fig. 6). However, their acuity is consistent with their visual features, namely nearly-complete panoramic visual fields and a visual streak (unpublished observation). Although many species, most notably primates, have their highest acuity (most densely packed ganglion cells) in a circular area called a fovea or area centralis, some species, like rabbits, have their best acuity spread in a narrow horizontal line across the retina, called a visual streak, giving them good acuity that spans the horizon. We reported many years ago (Heffner and Heffner 1992) that sound-localization acuity



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