

3 Focus: Sound-Localization Acuity Changes with Age in C57BL/6J Mice

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INTRODUCTION

C57BL/6J mice show a progressive high-frequency hearing loss beginning about 2 to 3 months of age (Chapters 13, 24, 28; Mikaelian, 1979). Because mammalian high-frequency hearing (i.e., above the 10-kHz limit of other vertebrates) evolved under strong selective pressure for sound localization, such a hearing loss should affect not only the ability of mice to detect sound, but also to localize it (R. Heffner and Heffner, 1992a; H. Heffner and Heffner, 1998). Specifically, high frequencies are necessary for two of the three basic locus cues: the binaural intensity difference of a sound at the two ears and the monaural pinna cues that arise from the directionality of the pinnae (the third cue being the binaural time difference, primarily a low-frequency cue). The two high-frequency cues require sounds that are effectively shadowed by the head and/or pinnae because low frequencies bend around small obstacles with little attenuation. Just how high an animal must hear to be able to use these cues depends on the size of its head and pinnae — the smaller the animal, the higher it must hear, which is why mice can hear above 80 kHz (H. Heffner and Masterton, 1980; Markl and Ehret, 1973). Thus, the loss of high-frequency hearing should have a detrimental effect on the ability of mice to localize sound, especially because they may not be able to compensate by relying on binaural time cues because the maximum interaural delay their small heads generate is so small (about 60 μ s).

We performed a study to observe the effect of age-related, high-frequency hearing loss in C57BL/6J mice by determining their sound-localization acuity at two different ages. An additional goal was to assess the ability of mice to localize brief sounds because previous research had only been able to demonstrate an ability to home in on sounds that were continuously repeated (Ehret and Dryer, 1984). Sound-localization thresholds and the ability to localize filtered noise bursts were determined for three C57BL/6J mice using an avoidance procedure involving suppression of drinking (for details, see H. Heffner and Heffner, 1995). The animals were then retested later, at which time their absolute thresholds for 16- and 32-kHz tones were also determined. The animals had free access to food in their home cages and received water during daily test sessions.

The mice were tested in a small, sound-transparent, wire mesh cage (15 \times 8 \times 10 cm) mounted on a camera tripod in the center of a carpeted, double-walled acoustic chamber, the walls and ceiling of which were lined with eggcrate foam to reduce sound reflections. The animals were trained to make steady contact with a water spout that came up through the floor of the cage in order to receive a slow but steady trickle of water dispensed via a syringe pump located outside the chamber. Drinking from the spout served to fix the animal's head in the center of a perimeter bar (1-m radius) on which loudspeakers were mounted. A typical test session lasted approximately 20 min, during which a mouse consumed 0.5 to 1.5 mL of water.

The mice were trained to drink in the presence of a 100-ms noise burst emitted from a loudspeaker to their right, but to break contact with the water spout whenever a sound was emitted

from a loudspeaker to their left in order to avoid a mild electric shock delivered through the spout. A 100-ms noise burst was presented once every 2.3 s from the left or right side on a quasi-random schedule in which about 22% of signals were from the left. An animal's response on each trial was determined by recording whether it was in contact with the spout during the last 200 ms of a trial (which was 1.8 s in duration). Breaking contact following a sound from the left was recorded as a hit, whereas breaking contact following a sound from the right was recorded as a false alarm.

Performance was quantified by determining hit and false alarm rates for a particular stimulus or angle in blocks of trials (typically 6 left and 25 right sounds). A performance measure was determined by correcting the hit rate according to the following formula: Performance = Hit rate - (Hit rate \times False alarm rate). Threshold, defined as the angle at which performance equaled 0.50, was determined by progressively reducing the separation between the loudspeakers until performance fell to chance (i.e., the hit and false alarm rates did not differ significantly).

Sound-localization thresholds were determined using a 100-ms broadband noise burst containing measurable frequencies from 3 to 80 kHz (6 to 48 kHz \pm 3dB), covering most of the hearing range of the domestic mouse (which, for sounds of 60 dB sound pressure level (SPL) ranges from 900 Hz to 79 kHz; Markl and Ehret, 1973). The intensity of the noise was varied randomly from 63 to 70 dB SPL to prevent the animals from using possible intensity differences between the loudspeakers as a cue.

A second test investigated the importance of high frequencies for localization by determining average performance for localizing low-pass filtered noise (48 dB/octave) at a fixed angle of 60° (\pm 30° left and right of midline). The five low-pass filter settings used (and the signal intensities re 20 μ Pa) were: 80 kHz (70 dB), 60 kHz (70 dB), 40 kHz (69 dB), 20 kHz (71.5 dB), and 10 kHz (67 dB). The filtered noise was presented with a 50-ms rise/fall time to avoid generating high-frequency onset and offset artifacts. (For the sound production and measurement equipment, see R. Heffner, Heffner, and Koay, 1995). Blocks of six left trials (and the associated right trials) were given at four different filter settings each day until 36 left trials had been given for each noise band. Finally, absolute thresholds for 16- and 32-kHz pure tones were determined at the end of testing to verify that the animals had a high-frequency hearing loss. In this case, the animals were trained to break contact with the spout whenever they heard a tone presented from a loudspeaker located in front of the cage (3 pulses, 400 ms on, 100 ms off, 10 ms rise/decay). The intensity of the tone was reduced in 5-dB increments until performance fell to chance and threshold was defined as the level at which performance equalled 0.50.

LOCALIZATION ACUITY

The mice had little difficulty learning to localize single, brief noise bursts. Initial training and detailed testing for localizing the 100-ms broadband noise required 21 and 25 sessions, respectively. During final threshold determination at 2.4 to 2.5 months of age, all three mice were performing well at angles of 90° or larger, and their mean threshold was 33° (Figure 3.1). Retraining and testing after more than a 3-month break required only 14 sessions, as the mice were now experienced with the test. Each animal's threshold, determined between 6.9 and 7.1 months of age, had increased on average by 13° to a new average of 46° (Figure 3.1). The increased thresholds cannot be attributed to an inability to hear the noise burst, nor to any deterioration in motivation, intellect, or general localization ability because performance at large angles remained normal.

LOCALIZATION OF LOW-PASS NOISE

Determination of the ability of the mice to localize low-pass noise bursts (60° separation) required six to eight sessions. Initial performance was determined at 2.5 to 2.7 months of age, and retesting took place at 7.2 to 7.4 months (Figure 3.2). Performance declined progressively as the high frequencies were removed, demonstrating the importance of high frequencies for azimuthal localization in mice. Animals A and B fell to chance when the filter was set at 20 kHz low-pass, and

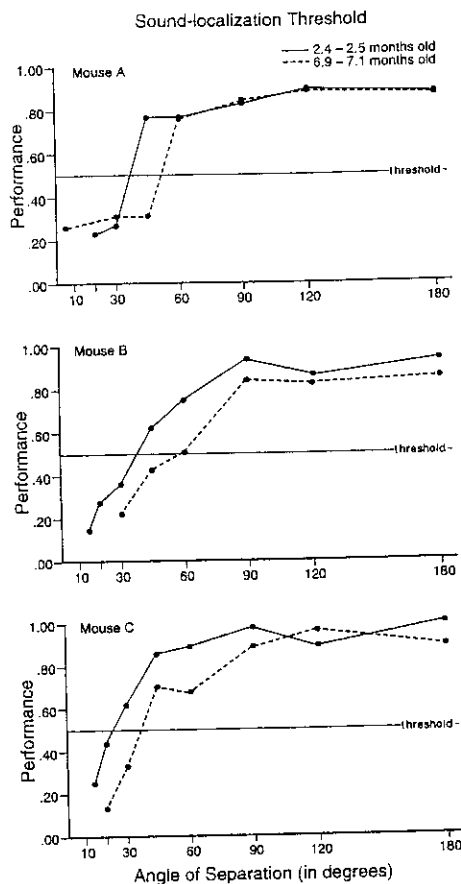


FIGURE 3.1 Sound-localization performances of the three mice at 2.4 to 2.5 months of age (solid lines) and 6.9 to 7.1 months (dashed lines). Thresholds increased with age from 37° to 51° for mouse A, 38° to 59° for mouse B, and 23.5° to 37° for mouse C.

all three mice were unable to localize above chance when the filter was set at 10 kHz low-pass although the noise burst was clearly audible. The animals showed a mild performance decrement as they aged, even when the signal contained high frequencies, presumably because a high-frequency hearing loss prevented the mice from making full use of them.

PURE-TONE THRESHOLDS

Detection thresholds at 16 and 32 kHz were completed when the mice were 7.7 months of age. Compared with NMRI mice (Markl and Ehret, 1973), the C57BL/6J mice showed hearing losses at 16 kHz of 16 to 54 dB and at 32 kHz of 52 to 59 dB (Table 3.1). Thus, these animals had an obvious hearing loss by 7.7 months of age.

DISCUSSION

TEST PROCEDURE

The use of an avoidance procedure in which an animal ceases responding when it detects a sound is a natural response that all three of the mice easily learned. Moreover, the animals could easily shift from one task (discriminating locus) to another (detecting tones) with little difficulty. Indeed,

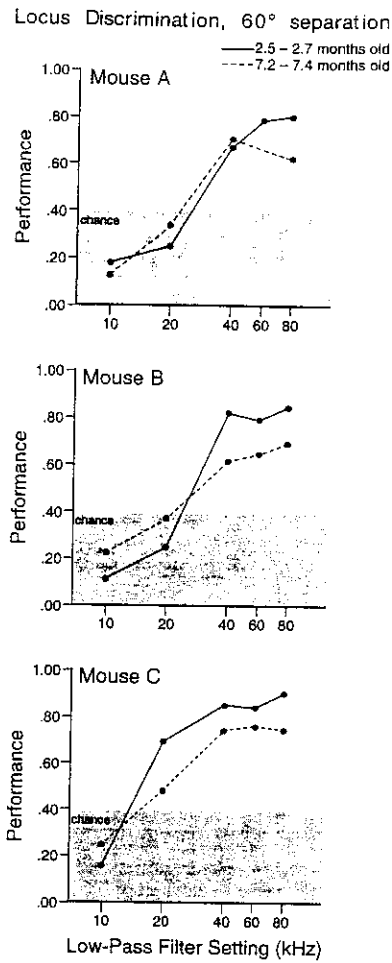


FIGURE 3.2 Sound localization performances of the three mice at 2.5 to 2.7 months of age (solid lines), and 7.2 to 7.4 months (dashed lines) with 100-ms low-pass filtered noise bursts presented 30° to the left or right of midline.

TABLE 3.1
Detection Thresholds (dB SPL) at 16 and 32 kHz
for Three C57BL/6J Mice at the End of Sound
Localization Testing (7.7 months old)

Strain	Frequency	
	16 kHz	32 kHz
NMRI (average) ^a	4 dB	22 dB
C57BL/6J (7.7 mo)		
Mouse A	20 dB	74 dB
Mouse B	41 dB	80 dB
Mouse C	58 dB	81 dB

^a Source: From Markl and Ehret, 1973. With permission.

this procedure has proven successful in assessing hearing in a wide variety of animals (e.g., H. Heffner and Heffner, 1995; 1998), and it probably accounts for the success in demonstrating that mice can localize brief sounds when previous studies have failed (Ehret and Dryer, 1984). Moreover, the combination of this task with the response of eating or drinking from a spout, which fixes an animal's head in the sound field, makes it ideal for auditory testing.

SOUND LOCALIZATION CHANGES ACCOMPANYING AGING

From 2.7 to 7.4 months of age, the average localization thresholds of the mice increased from 33° to 46°. Because this strain of mice is known to show a progressive high-frequency hearing loss beginning at 2 to 3 months of age, and because the mice tested here demonstrated a large high-frequency hearing loss at 7.7 months, it is probable that the increased locus thresholds were due to their hearing loss. That the mice did not suffer from any general deterioration in localization ability is suggested by the observation that their localization performance at large angles was essentially normal (Figure 3.1).

The loss of high frequencies does not affect left-right sound localization acuity in all species. For example, humans with high-frequency sensorineural hearing loss retain good left-right acuity, presumably because binaural time differences, which use low frequencies, are sufficient to maintain good localization (Colburn, Zurek, and Durlach, 1987; Noble, Byrne, and Lepage, 1994). Similarly, the left-right localization acuity of chinchillas is not degraded by filtering out high frequencies, as it is in mice. However, it should be noted that both humans and chinchillas require high frequencies to discriminate the elevation of sound and to prevent front-back reversals (Belendiuk and Butler, 1975; R. Heffner et al., 1994; 1995). The fact that mice require high frequencies for left-right localization, however, suggests that they may not use the binaural time-difference cue for locus. This would not be unique because at least one other small mammal, the big brown bat, appears to have relinquished the use of the binaural time cue, relying solely on interaural intensity differences and monaural pinna cues for passive localization (Koay et al., 1997).

COMPARISON OF MICE TO OTHER MAMMALS

The 33° mean threshold for these mice at 2.5 to 2.7 months of age is larger than that of most mammals, but comparable to that of gerbils, kangaroo rats, horses, and cattle (H. Heffner and Heffner, 1998). Although some investigators have suggested that there may be small high-frequency losses in C57BL/6J mice at 2 months of age (Li and Borg, 1991), it is likely that the explanation for the comparatively poor locus acuity in these mice lies in the relationship between hearing and vision — the primary function of sound localization is to direct the eyes to the source of a sound for visual examination (R. Heffner and Heffner, 1992c; Koay et al., 1998). Just how accurately the ears must direct the eyes depends on the width of an animal's field of best vision. Specifically, animals with narrow fields of best vision (e.g., humans and elephants) require better locus acuity than animals with broad fields of best vision (e.g., kangaroo rats and cattle). Because the density of the ganglion cells in the mouse retina indicates that it has a relatively broad field of best vision of 114° (based on retinal ganglion-cell density gradients, R. Heffner, unpublished), its comparatively poor localization acuity is not unexpected.

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