Overview of Methods for Assessing the Mouse Auditory System

Numerous types of research require auditory testing of mice. These include: (1) the study of models for deafness or hearing loss; (2) evaluation of interesting strains and mutants with alterations in biochemistry, physiology, neurotransmitters, and other parameters that carry auditory implications; (3) confirmation that mice can hear auditory stimuli used in experiments (e.g., an auditory conditioned stimulus, stressor); and (4) screening large numbers of mice in genetic studies (e.g., mutagenesis). Auditory testing may also be included (5) as one aspect of a battery of general tests.

The three approaches discussed in the subsequent units of this chapter—measurement of the auditory brainstem response (ABR; *UNIT 8.21B*), measurement of distortion product otoacoustic emissions (DPOAEs; *UNIT 8.21C*), and the lick suppression behavioral test (*UNIT 8.21D*)—are complementary but differ in the time required for testing as well as in regard to the goals of the tests. Obtaining a threshold with the ABR is the quickest and technically easiest way to assess auditory sensitivity in mice. DPOAEs, which are more difficult to measure, are indicative of outer hair cell (OHC) function, providing information as to the source of hearing loss. Both of these physiological measures reflect cochlea sensitivity to acoustic stimuli (rather than hearing per se) and require anesthetization. They are, therefore, one or two steps removed from auditory behavior and perception. In contrast, lick suppression requires the mouse to respond behaviorally to an auditory stimulus, thus allowing direct determination of a psychophysical threshold.

ISSUES TO CONSIDER WHEN STUDYING MICE

Inbred strains of mice differ widely and often dramatically with regard to a myriad of phenotypic traits. Some traits may have obvious implications for hearing (e.g., cochlear histopathology), but more subtle differences in physiology, immune system activity, drug reactions, and behavior may affect hearing, auditory behaviors, and/or test outcomes as well. For this reason, relevant literature on the strain or mutant should be investigated. It is important that the complete nomenclature of the strain be identified, because even substrains may differ in important ways. For example, the CBA/CaJ and CBA/J strains both maintain good hearing as they age, but CBA/CaJ mice are longer-lived, more robust, and behaviorally more active (a real consideration in behavioral experiments). Thus, simply identifying a strain as "CBA" is not adequate. Substrains of 129, C57, and others differ with respect to hearing loss as well (see Willott, 2001, and *http://www.jax.org/phenome*). Inbred strains may have other nonauditory anomalies that are not obvious but which could be important in some contexts. For example, CBA/J and several C3H lines are commonly used strains that possess a retinal degeneration gene that results in blindness. One would never suspect this merely by casually observing these mice, but the implications for behavioral discrimination involving visual cues, circadian light cues, and the like are significant.

Whereas strains differ greatly from one another, all mice of an inbred strain are genetically identical and homozygous at all loci (F1 hybrid strains derived from two inbred strains are also genetically identical to one another, but are not homozygous at all loci). Thus, all variance among mice of an inbred strain is due to nongenetic factors. Surprisingly, within-strain phenotypic variance can be substantial even when environmental variables are well controlled. In some cases, the impact of environmental, hormonal, in utero, postnatal, social, and/or behavioral events may actually be greater in inbred mice as compared with genetically heterogeneous mice, because the latter may have a broader range of mechanisms that can counteract an environmental insult to dampen variance. Thus, while all strain members will exhibit a phenotype, it is not valid to assume that they will, necessarily, be phenotypically similar to one another in all respects. For example, essentially all members of strains such as C57BL/6J or DBA/2J (which possess the *Ahl* gene for hearing loss) will exhibit progressive sensorineural hearing loss; however, the time course will vary within some range among individuals.

Contributed by James F. Willott
Current Protocols in Neuroscience (2005) 8.21A.1-8.21A.2

WHAT FREQUENCIES SHOULD BE TESTED?

The selection of frequencies to use for testing hearing in mice is important. Many mice can hear tones approaching 100 kHz (Fay, 1988), although this is often not the case for inbred lines. Assuming that a mouse has an upper frequency range of 100 kHz, if the highest tone frequency tested is 32 kHz, this would be between 1 and 2 octaves below the mouse's upper range (50 kHz is one octave down from 100 kHz; 25 kHz is 1 octave down from 50 kHz). By analogy, the upper frequency range for humans is about 20 kHz, and a tone that is 1 to 2 octaves below this would be about 6 to 7 kHz. Most studies of human hearing do not use frequencies higher than this, so 32 kHz would be a reasonable high-frequency cut-off for many studies of mouse hearing, falling roughly within standards used for human experimentation. However, the octave range in humans is broader than it is in mice, so this comparison may be questioned (i.e., perhaps, with respect to the low range of hearing, frequencies higher than 32 kHz are warranted). Another way to view the issue is with respect to the representation of frequency along the basilar membrane. In mice, the 32 kHz region of the cochlea is about 25% of the distance from base to apex (Ehret, 1983; Bohne et al., 2001). This corresponds to about 8 kHz in humans (e.g., Nadol, 1981).

The author's view is that 32-kHz tones are adequate for most mouse experiments. However, the addition of higher frequencies is encouraged if indicated by the goals of the study (e.g., a full "audiogram" or measure of sensitivity to ultrasonic vocalizations) and/or hearing capacity of the mice used (e.g., mice with excellent high-frequency hearing). Many commercially available headphones or tweeters produce reasonable-quality tones of 50 to 60 kHz, and some manufacturers of behavioral testing or ABR recording equipment designed for use with rodents provide speakers with ultrasonic ranges.

APPROVAL OF PROTOCOL

All procedures must have prior approval by the appropriate committee on animal experimentation. In the United States, this is the Institutional Animal Care and Use Committee (IACUC), which also assures that federal guidelines are met.

LITERATURE CITED

Bohne, B.A., Harding, G.W., and Ou, H.C. 2001. Preparation and evaluation of the mouse temporal bone. *In* Handbook of Mouse Auditory Research: From Behavior to Molecular Biology (J.F. Willott, ed.) pp. 171-188. CRC Press, Boca Raton, Fla.

Ehret, G. 1983. Peripheral anatomy and physiology II. *In* Auditory Psychobiology of the Mouse (J.F. Willott, ed.) pp. 169-200. Charles C. Thomas, Springfield, Ill.

Fay, R.R. 1988. Hearing in Vertebrates: A Psychophysics Databook, Hill-Fay, Winnetka, Ill.

Nadol, J.B., Jr. 1981. The aging peripheral hearing mechanism. *In* Aging: Communication Processes and Disorders (D.S. Beasley and G.A. Davis, eds.) pp. 63-85. Grune & Stratton, New York.

KEY REFERENCES

Parham, K., Sun, X.-M., and Kim, D.O. 2001. Noninvasive assessment of auditory function: Distortion product otoacoustic emissions and auditory brainstem responses. *In* Handbook of Mouse Auditory Research: From Behavior to Molecular Biology (J.F. Willott, ed.) pp. 37-58. CRC Press, Boca Raton, Fla.

More on electrophysiological methods.

Heffner, H. and Heffner, R.S. 2001. Behavioral assessment of hearing in mice. *In* Handbook of Mouse Auditory Research: From Behavior to Molecular Biology (J.F. Willott, ed.) pp. 19-30. CRC Press, Boca Raton, Fla.

More on behavioral tests.

Willott, J.F. (ed.). 1983. The Auditory Psychobiology of the Mouse, Charles C. Thomas Publishers, Springfield, Ill.

Summary of early research.

Willott, J.F. (ed.). 2001. Handbook of Mouse Auditory Research: From Behavior to Molecular Biology, CRC Press, Boca Raton, Fla.

Summary of recent research.

Contributed by James F. Willott
University of South Florida and
The Jackson Laboratory
Tampa, Florida