Heffner, H. E. and Heffner, R. S. (2001). Behavioral assessment of hearing in mice. In: J.F. Willott (Ed.) *Handbook of Mouse Auditory Research: From Behavior to Molecular Biology*. (pp. 19-29). CRC Press: Boca Raton FL.

Behavioral Assessment of Hearing in Mice

Henry E. H0.00 0h1Hi0 Tw(. (p)Tjehavmmp

Abstract

Five conditioned 2

assessing hearing disorders, it is necessary to employ behavioral procedures to obtain valid measures of auditory function.

The behavioral procedures available for assessing hearing in animals can be divided into two types: those that train an animal to respond to sound using conditioning procedures, and those that make use of unconditioned or reflexive responses to sound. Conditioning procedures have been considered to be more sensitive than reflexive measures as the animals are carefully trained to be reliable observers. Moreover, these procedures are easily adapted to testing the ability of animals to discriminate between, as well as to detect sounds. However, conditioning procedures can be difficult to use and an animal may require lengthy training before it is ready for testing. This has led to the development of procedures involving unconditioned reflexes that are simpler to administer and involve no training of the animal. Although most tests that make use of unconditioned reflexes are limited to determining an animal's ability to hear loud sounds, one procedure, prepulse inhibition, is able to determine both an animal's ability to hear low-level sounds and to discriminate between sounds.

The essential feature of any auditory test is that the animal makes a clearly defined that may occur when sounds are turned on and off abruptly.

There are a number of behavioral procedures available for assessing animal hearing (cf., Klump et al., 1995) and it is the purpose of this chapter to describe and evaluate those that have been, or could be, used with mice. It is not our intention to provide a manual on how to conduct these tests, but to describe them in sufficient detail that one might choose between them. Anyone interested in using a particular procedure should contact investigators who use them as no written description can cover all details, and procedures are constantly being updated and refined.

2. Procedures using conditioned responses

It is not uncommon to divide conditioning procedures into those using operant (or instrumental) conditioning, in which an animal emits a response to obtain a reward or avoid a punisher, and those using classical conditioning in which a stimulus elicits a response. However, whether or not a particular procedure is true classical conditioning is a technical issue that does not affect its use in sensory testing and the main issue in this chapter is the ease of use and validity of the results.

All conditioning procedures involve either a reward, a punisher, or both. Rewards used for mice have included sweetened water and milk (Birch et al., 1968; Sidman et al., 1966). However, water by itself works well and can be reliably delivered with a commercial syringe pump or water dipper (e.g., Heffner et al., in press; Prosen et al., 2000). Electric shock is commonly used in both avoidance and classical conditioning with the levels kept relatively low because high levels can interfere with performance by causing the animals to develop a fear of the test apparatus (Heffner and Heffner, 1995).

2.1. Conditioned suppression/avoidance

In devising a psychophysical procedure for use with animals, it is helpful to choose a task that utilizes an animal's natural responses thus making the task easier to learn. One response common to all mammals is to suppress ongoing behavior (i.e., freeze) upon detection of a stimulus that might signal danger. The suppression of behavior as a procedure for testing hearing was originally developed for mice and has since been adapted for auditory testing in other animals (Heffner and Heffner, 1998; Ray, 1970; Sidman et al., 1966). The current procedure consists of allowing an animal to make steady contact with a water spout in order to obtain water and then training it to momentarily break contact whenever it hears a sound that signals

often fail to respond to a tone if it was grooming or otherwise engaged even though it was in the listening compartment.

A simpler version of the go/no-go procedure, one that dispenses with the listening compartment, has been used successfully to assess the ability of mice to perform a number of auditory discriminations (Ehret, 1975a; Markl and Ehret, 1973). In this procedure, a mouse is placed in a small test cage with a water spout and loudspeaker located in one corner. Variation in the sound pressure level is minimized by confining the animal to a small area, in this case a 10 x 10-cm platform, and presenting tones when the animal is facing the loudspeaker. Tones are presented at random intervals and the animal is required to maintain contact with the spout for 3 s while a tone is on in order to obtain a water reward. Because the animals reportedly do not lick the water spout for more than 2 s in the absence of a tone, false positives are not a problem. Sessions last approximately 10 min during which 20 tone presentations are made.

This procedure has been used to assess absolute thresholds, frequency and intensity difference limens, temporal integration, and masking (Ehret, 1983). Because it is not necessary to train the animal to enter a listening compartment, initial conditioning is accomplished in 8 days. However, the procedure works best with tame mice, that is, animals that have been handled so they are not overly shy or nervous. As a result, not all animals can be successfully the animal to wait an additional 5 s before testing resumes. Finally, the detection rate is corrected for false alarms. Unfortunately, these modifications do not seem to have solved the problems of the earlier version as the animal's head is still not fixed within the sound field and the procedure results in false alarm rates of 20% and higher, well above the rate generally considered acceptable (Stebbins, 1970). Moreover, this version involves a complicated training procedure that requires a month or more before an animal is ready to test, making it the slowest procedure of all.

2.3 Conditioned eyeblink

Auditory thresholds have been obtained in mice by conditioning them to close their eyes when a sound signaling impending shock is presented (Ehret 1976a). It is important to note, however, that this is not the standard eyeblink reflex used by others as the aversive stimulus is delivered to an animal's feet, not to one of its eyes (c.f., Martin et al., 1980). Thus, the response of closing the eyes is most likely part of a larger pattern of behavior in which the animal reacts to the anticipated shock.

For testing, the animal is placed in a small cage with a floor constructed of metal bars. Tones are presented at random intervals for a duration of 1 s and are followed immediately by a brief electric shock delivered through the cage floor. Sessions last about 10 min during which 20 trials are given. The interval between trials varies from 3 to 60 s, depending on the behavior of the animal because good responses can only be obtained when the animal is not moving. This procedure is a simple method for assessing hearing, and those mice that are able to learn it respond reliably after 8 training sessions.

The eyeblink procedure can give good results, and audiograms generated by it are virtually identical to those obtained using the go/no-go procedure (Markl and Ehret, 1973). In addition, it has the advantage that it is not necessary to deprive an animal of food or water. However, it requires the animal to sit motionless during testing and only about 50% of mice can be trained to respond reliably (e.g., Ehret, 1976a). Moreover, the response is determined subjectively by the experimenter and does not lend itself to automation, opening the possibility of observer bias. Finally, the procedure has not been used to test auditory discriminations, most likely because discrimination tests tend to have high false positive rates and this procedure has no means to control them.

cage 120° apart. The animal was then required to approach the active loudspeaker and contact the water spout located in front of it. However, the animals never performed the task well and failed to respond if the sound was turned off before they reached the water spout. Even if the sound was left on until they completed their response, they often did not directly approach the loudspeaker.

It is not clear why this procedure did not work well with mice as it has been used successfully to assess sound localization in other species, including rats and gerbils (e.g., Heffner and Heffner, 1988b; Masterton et al., 1975). As used by others, the procedure insures that an electric shock that is turned on 0.5 s after tone onset. Because the GSR occurs 0.5-3.5 s after tone onset, a GSR response to the tone itself can only be observed for tone presentations not followed by shock. Therefore, tones are followed by shock 40% of the time to maintain conditioning, with the tone-only trials analyzed to determine if a response occurred. Control trials are used in which no tone or shock is presented in order to obtain a measure of the animal's false alarm rate. The tones are attenuated to obtain the lowest intensity that yields a detectable response.

An audiogram for mice, derived by taking the lowest 10% of the thresholds obtained from a group of 50 mice, showed reasonable sensitivity at low and middle frequencies, although they appeared to be less sensitive to high frequencies than indicated by another audiogram that used the same strain of mice (CBA/J). This difference raises the possibility that GSR conditioning may not give accurate results at high frequencies (cf., Berlin, 1963; Birch et al., 1968). In addition, the animals showed extreme variation, differing by as much as 80 dB at some frequencies. Moreover, the animals failed to condition to a tone approximately 25% of the time and pregnant and estrous females proved too variable to be used. Thus, as noted by the investiga2l0.06ltho37.9ucj22.92800 TD.00 0.E discriminate between sounds. However, there is one unconditioned procedure which may have overcome these limitations, prepulse inhibition, which takes advantage of the fact that the acoustic startle reflex to a loud sound can be modified by preceding it with another sound.

3.1 Acoustic startle reflex

Mice, like other mammals, show an unconditioned motor reaction to sudden loud sounds, a response referred to as the acoustic startle reflex (e.g., Hoffman and Ison, 1980). The sound used to produce the startle reflex must be loud (e.g., 100 dB re 20 µPa) and have a near instantaneous onset time as sounds with onset times much greater than 10 ms may not elicit a startle. In addition, the startle reflex is best elicited when the animal is sitting quietly as a moving animal may not show a startle response.

The reflex is measured by placing an animal in cage and presenting a startle sound at random intervals when the animal is not moving (e.g., Parham and Willott, 1988). The response of the animal to the startle sound is detected with an accelerometer attached to the test cage. A variety of startle sounds have been used including noise (e.g., 10-25 ms noise burst, 100-115 dB, 1-5 ms rise/fall time) and tones (e.g., 4-24 kHz tone burst, 70-110 dB, 10 ms duration, 1-ms

rise0 0 d0.4400 TD(riset0.00 placing an animal in cage a-ms rgBT72.0000 388.8000 TD0.0600 Tc-0.0600 Tw(1-5 ms rise)Tj53.2800 0.0000 T

those found using conditioned response procedures, at least for the frequencies in the midrange of the audiogram (Fechter et al., 1988). However, the issue of validity is best settled by comparing thresholds obtained with this procedure with those obtained for the same sounds is a measure of absolute threshold. In other words, the absence of a response does not indicate that the animal cannot hear the sound, only that it is not responding to it. The best demonstration of the usefulness of pinna movements elicited by less intense sounds has been in studies of the development of hearing in young mice (Ehret, 1976a; 1977). However, it may now be possible to study hearing even in young mice using other techniques such as the acoustic startle reflex modification procedure and even, perhaps, the conditioned suppression/avoidance procedure.

3.4 Freezing response

An animal that is moving about may stop or freeze when it hears an unexpected sound. This reaction has been used to demonstrate hearing in mice under 12 days of age, at which time the pinna detaches from the scalp and pinna movements can be observed (Ehret, 1976a, 1977). As with the pinna movements discussed above (section 3.3), the freezing response is probably not a measure of absolute sensitivity although it can give useful information in the absence of other measures. More sensitive procedures, such as the acoustic startle reflex modification procedure or conditioned suppression/avoidance procedure should be tried first before using this procedure.

3.5 Galvanic skin response (GSR)

As previously noted (section 2.5), the galvanic skin response (GSR) occurs as an unconditioned response to loud sounds. The unconditioned GSR has been used to study the

the most potential for auditory testing. Not only is this procedure capable of demonstrating an animal's ability to detect sounds of low intensity, but it can also be used to determine the ability to discriminate between sounds. All that is required is to verify that the thresholds obtained with this procedure are as sensitive 0 TD(re)Tj4.2500 0.0000 TD(is)Tj8.0400 0.00000 D(pro)Tj18.9600 0.000.0j56 4e00 speed of testing would be to optimize the test apparatus by determining the size and shape of the water spout and water reward rate that work best for mice.

The other behavioral procedure that holds great promise is prepulse inhibition. Because this procedure makes use of an unconditioned reflex, it is potentially the fastest of all procedures for assessing hearing in mice a

musculus). J. Am. Audiol. Soc. 1, 179-184.

Ehret, G., 1976b. Temporal auditory summation for pure tones and white noise in the house mouse (*Mus musculus*). J. Acoust. Soc. Am. 59, 1421-1427.

Ehret, G., 1977. Postnatal development in the acoustic system of the house mouse in the light of developing masked thresholds. J. Acoust. Soc. Am. 62, 143-148.

Ehret, G., 1983. Chapter 2, Psychoacoustics. In J. F. Willott (Ed.) The Auditory Psychobiology of the Mouse. Charles C. Thomas Pub.

Ehret, G., Dreyer, A., 1984. Localization of tones and noise in the horizontal plane by unrestrained house mice (*Mus musculus*). J. Exp. Biol. 109, 163-174.

Fechter, L.D., Sheppard, L., Young, J.S., Zeger, S., 1988. Sensory threshold estimation from a continuously graded response produced by reflex modification audiometry. J. Acoust. Soc. Am. 84, 179-185.

Francis, R. L. 1979., The Preyer reflex audiogram of several rodents and its relation to the "absolute" audiogram in the rat. J. Aud. Res. 19, 217-233.

Hack, M.H. 1968. The developmental Preyer reflex in the sh-1 mouse. J. Aud. Res. 8, 449-457.

Heffner, H.E., Heffner, R.S., 1986. Effect of unilateral and bilateral auditory cortex lesions on the discrimination of vocalizations by Japanese macaques. J. Neurophysiol. 56, 683-701.

Heffner, H. E., Heffner, R. S., 1995. Conditioned avoidance. In G. M. Klump, R. J. Dooling, R. R. Fay, and W. C. Stebbins (Eds.), Methods in Comparative Psychoacoustics. Birkhäuser:Basel, pp. 73-87.

Heffner, H.E. Heffner, R.S. 1998. Hearing. In G. Greenberg and M. M. Haraway (Eds.), Comparative Psychology, A Handbook. Garland: New York, pp. 290- 303.

Heffner, H., Masterton, B., 1980. Hearing in glires: domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. J. Acoust. Soc. Amer. 68, 1584-1599.

Heffner, R.S., Heffner, H.E., 1983. Hearing in large mammals: Horses (*Equus caballus*) and cattle (*Bos taurus*). Behav. Neurosci. 97, 299-309.

Heffner, R.S., Heffner, H.E., 1988a. Sound localization acuity in the cat: Effect of azimuth, signal duration, and test procedure. Hear. Res. 36, 221-232.

Heffner, R.S., Heffner, H.E., 1988b. Sound localization and the use of binaural cues by the gerbil (*Meriones unguiculatus*). Behav. Neurosci. 102, 422-428.

Heffner, R.S ., Heffner, H.E., 1992. Hearing in large mammals: Sound-localization acuity in cattle (*Bos taurus*) and goats (*Capra hircus*). J. Comp. Psychol., 106, 107-113.

Heffner, R.S., Koay, G., Heffner, H.E., In press. Sound-localization acuity changes with age in C57BL/6J mice. In: J.F. Willott (Ed.) Handbook of Mouse Auditory Research: From

Behavior to Molecular Biology. CRC Press,

Hoffman, H., Ison, J. R., 1980. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol. Rev. 87, 175-189.

Hood, L.J., 1998. Clinical Applications of the Auditory Brainstem Response. San Diego: Singular Publishing Group.

Hood L.J, Berlin C.I, Allen, P., 1994. Cortical deafness: A longitudinal study. J. Amer. Acad. Audiol. 5, 330-342.

Ison, J.R., Agrawall, P., 1998. The effect of spatial separation of signal and noise on masking in the free field as a function of signal frequency and age in the mouse. J. Acoust. Soc. Am. 104, 1689-1695.

Ison, J.R., Agrawal, P. Pak, J., Vaughn, W.J., 1998. Changes in temporal acuity with age and with hearing impairment in the mouse: A study of the acoustic startle reflex and its inhibition by brief decrements in noise level. J. Acoust. Soc. Am. 104, 1696-1704.

Ison, J.R., In press. Prepulse inhibition. In: J.F. Willott (Ed.) Handbook of Mouse Auditory Research: From Behavior to Molecular Biology. CRC Press, pp.

Kulig, J., Willott, J.F., 1984. Frequency difference limens of C57BL/6 and DBA/2 mice: Relationship to auditory neuronal response properties and hearing impairment. Hear. Res. 16, 169-174.

Klump, G.M., Dooling, R.J. Fay, R.R. Fay, Stebbins, W.C. (Eds.), 1995. Methods in Comparative Psychoacoustics. Birkhäuser: Basel.

Markl, H., Ehret, G., 1973. Die Hörschwelle der Maus (*Mus musculus*). Eine kritische Wertung der Methoden zur Bestimmung der Hörschwelle eines Säugetieres. Z. Tierpsychol. 33, 274-286.

Martin, G.K., Lonsbury-Martin, B.L., Kimm, J., 1980. A rabbit preparation for neuro-behavioral auditory research. Hear. Res., 2, 65-78.

Master, R.G., Thompson, G.C., Bechtold, J.K., RoBards, M.J., 1975. Neuroanatomical basis of binaural phase-difference analysis for sound localization: A comparative study. J. Comp. Physiol. Psychol. 89, 379-386.

Moore, B.C.J., 1997. An introduction to the psychology of hearing, 4th ed. New York: Academic Press.

Parham, K., Willott, J.F., 1988. Acoustic startle response in young and aging C57/BL/6J and CBA/J mice. Behav. Neurosci. 102, 881-886. and 80 kHz by shuttle box training. *J. Aud. Res*. 19, 37-40.

Prosen, C.A., Bath, K.G., Vetter, D.E., May, B.J., 2000. Behavioral assessments of auditory sensitivity in transgenic mice. J. Neurosci. Methods 97, 59-67.

Ray, B.A., 1970. Psychophysical testing of neurologic mutant mice. In W.C. Stebbins (Ed.), Animal Psychophysics. New York:Appleton, Century, Crofts.

Schleidt, W.M., Kickert-Magg, M., 1979. Hearing thresholds of albino house mouse between 1 and 80 kHz by shuttle box training. J. Aud. Res. 19, 37-40.

Sidman, M., Ray, B.A., Sidman, R.L., Klinger, J.M., 1966. Hearing and vision in neurological mutant mice: A method for their evaluation. Exptl. Neurol. 16, 377-402.

Starr A, Picton TW, Sininger YS, Hood LJ, Berlin CI., 1996. Auditory neuropathy. Brain 119, 741-753.

Stebbins, W.C., 1970. Studies of hearing and hearing loss in the monkey. In: W. C. Stebbins (Ed.), Animal Psychophysics, Appleton-Century-Crofts, New York, pp. 41-66.

Thompson, G., Heffner, H., Masterton, B., 1974. An automated localization chamber. Behav. Res. Methods Instr. 6, 550-552.

Willott, J.M., Turner, J.G., 1999. Prolonged exposure to an augmented acoustic environment ameliorates age-related auditory changes in C57BL/6J and DBA/2J mice. Hear. Res. 135, 78-88.

Woodworth, R.S., Schlosberg, H., 1965. Experimental Psychology, Revised Edition. New York: Holt, Rinehart and Winston.

Zheng, Q.Y., Johnson, K.R., Erway, L.C., 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130, 94-107.