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Behavioral Assessment of Hearing in Mice

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Abstract

Five conditioned and five unconditioned response procedures for assessing hearing in mice are reviewed. The five conditioned response procedures are conditioned suppression/avoidance, go/no-go, eyeblink conditioning, avoidance conditioning, approaching the source of a sound, and GSR conditioning. Of these, the conditioned suppression/avoidance procedure is the most effective as it has the shortest training time, allows the most trials to be given, holds the animal's head fixed in the sound field, and is successful with animals considered difficult to test. The five unconditioned procedures are the acoustic startle reflex, prepulse inhibition, pinna movements, freezing response, and GSR. Of these, the prepulse inhibition procedure holds the most promise for auditory testing—unlike other unconditioned response procedures, it is it capable of demonstrating an animal's ability to detect low intensity sounds as well as its ability to discriminate between sounds.

1. Introduction

The domestic house mouse (*Mus musculus*) has become an important animal model for the study of genetic hearing loss. Not only are there strains with naturally occurring hearing impairments, but genetically engineered mice can be used to identify the specific genes involved in hearing disorders. To fully understand the effects of genetic mutations on hearing, however, it is necessary to determine with some precision the hearing abilities of these mice. This includes not only absolute thresholds, but other aspects of hearing such as frequency and intensity discrimination, the effect of masking, and sound localization acuity.

There are two general approaches to assessing hearing in mice: electrophysiological and behavioral. A popular way of assessing hearing in mice is to use an electrophysiological measure, specifically the auditory brainstem response or ABR (e.g., Zheng et al., 1999). The popularity of this technique stems from the fact that it is relatively easy to learn and can provide rapid results, with measurements usually taking no more than an hour or so. However, the ABR is actually a measure of neural synchrony, not auditory sensitivity, and at best can only be used to infer absolute sensitivity (Hood, 1998). In addition, it cannot provide information regarding an animal's ability to discriminate sounds. In the clinic, the ABR is useful in diagnosing auditory disorders, but has not supplanted behavioral tests of hearing. Indeed, it is known on occasion to significantly overestimate and underestimate the effect of auditory malfunctions on sensory thresholds (Hood et al., 1994; Starr et al., 1996). Thus, although the ABR is a useful tool in 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 response when one stimulus is presented and a different response when either no stimulus or a different stimulus is presented. Although it is possible to determine an animal's response through direct observation, it is preferable to use an automated recording system to rule out the possibility of observer bias. The animal should respond reliably to obviously suprathreshold stimuli, and decreasing the stimulus level or the difference between the stimuli must eventually result in chance performance, indicating that the animal is not using some extraneous cue to perform the task. In addition, it is commonly observed in conditioning procedures that the thresholds of naive animals improve as the animals become experienced observers, suggesting that it may be necessary for animals to learn to listen for sounds near threshold (Stebbins, 1970). Finally, the ideal test is one that is easy to use, works with all individuals, and provides reliable and valid results in a short period of time.

The acoustic environment is also important. Test procedures should be capable of fixing an animal's head within the sound field so that the sound reaching the ears can be precisely specified. Reflections from the walls of a cage should be avoided by constructing the test cage of sound-transparent material, such as wire mesh, and any necessary response or reward mechanisms kept small and out of the path of the sound. For example, a water reward can be delivered through a thin vertical water spout that comes up through the cage floor and is thus well below the level of the animal's ears (Heffner and Heffner, 1995). Sounds to be detected or discriminated should be carefully checked for distortion as well as for onset and offset artifacts 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 impending shock. Because this procedure involves avoidable shock, we have previously referred to it as “conditioned avoidance” to distinguish it from earlier conditioned suppression procedures that used unavoidable shock (cf., Heffner and Heffner, 1995; Sidman et al., 1966). However, because the suppression of ongoing activity is the key feature, we refer to it here as “conditioned suppression/avoidance.”

To determine absolute thresholds, a thirsty mouse is placed in a test cage where it drinks from a water spout that delivers water as long as the animal is in contact with the spout. Next, a tone is presented for 2 s after which a mild electric shock is delivered through the water spout. The animal quickly learns to associate the tone with the shock and breaks contact with the spout whenever it detects the tone in order to avoid the shock. The use of avoidable, as opposed to unavoidable shock, significantly increases the number of trials that can be given.

Test sessions are divided into 2-s trials with 1.5-s intertrial intervals. Tone or “warning” trials are presented on a random schedule with approximately 22% of the trials containing a tone. The response of an animal is scored as a “hit” if the animal breaks contact during the last 150 ms of a trial that contains a tone. Breaking contact during a trial that does not contain a tone (a “safe” trial) is scored as a “false alarm” (FA). The hit rate is then corrected for the false alarm rate; one common correction is: Corrected Hit Rate = Hit Rate - (Hit Rate x FA Rate). Another is: Corrected Hit Rate = Hit Rate - FA Rate. The intensity of the tone is lowered until the animal’s performance falls to statistical chance (i.e., the hit and false alarm rates are not significantly different) and threshold is defined as the intensity yielding a corrected hit rate of 0.50. Sessions last approximately 20 minutes during which 25-30 warning trials can be given.

There are four features of the conditioned suppression/avoidance procedure that give it an advantage over other conditioning procedures. First, the animal’s head is held in a fixed position by requiring it to make contact with the water spout. This not only allows the sound pressure level at the animal’s ears to be specified with precision (e.g., ±1 dB), but is essential for sound localization tests in which the azimuth of a sound source relative to an animal’s head must be specified. Second, the procedure incorporates a “ready” or “observing” response in that trials are not presented unless the animal is in contact with the spout during the preceding intertrial interval. This requirement avoids presenting trials when an animal is grooming or otherwise not attending to the task. Third, the false alarm rate is easily controlled by changing the shock level and/or the reward rate. Thus, a high false alarm rate can be quickly lowered by reducing the level of the shock and/or changing the water flow rate. Finally, the procedure is easily adapted for testing any auditory discrimination, such as frequency discrimination, intensity discrimination, and sound localization acuity and can even be used to assess an animal’s ability to categorize sounds (e.g., Heffner and Heffner, 1986; Heffner et al., in press). Tests of auditory discrimination are conducted by presenting one stimulus during safe trials (e.g., a sound from the right) and a different stimulus during warning trials (e.g., a sound from the left).

In summary, conditioned suppression/avoidance is a simple procedure for an animal to learn and has been used to test hearing in more species than any other method, including wild house mice (Heffner and Heffner, 1998; Heffner and Masterton, 1980). Given a water spout of appropriate size and height for mice and a reliable syringe pump to deliver the 0.5-2 ml of water that a domestic mouse will drink in one session, it takes 5-10 sessions for a naive mouse to become accustomed to the test cage and learn to reliably respond to suprathreshold stimuli. The time required for testing depends on the number of data points to be collected and thresholds may be most efficiently obtained by using a tracking procedure (Ray, 1970; Sidman et al., 1966).

2.2 Go/no-go

The standard procedure for assessing hearing in humans is to ask subjects to raise a hand when they hear a tone. The equivalent approach in animals is a go/no-go procedure that requires an animal to wait patiently until a stimulus is presented and then respond within a fixed amount of time.

The first go/no-go procedure to obtain a complete audiogram for mice used a test cage that was divided in half by a barrier (Birch et al., 1968). The animals were required to wait underneath a loudspeaker on one side of the barrier (a "listening" compartment) and to cross over to the other side and press a lever within 10 s of tone onset in order to receive a sweetened water reward. Trials were presented at random intervals and the intensity of the tone was reduced to obtain a threshold. Requiring the animals to wait under the loudspeaker positioned them in the sound field, although the intensity of the sound at the animal's ears varied depending on where the mouse stood and whether or not its ears were directed up at the loudspeaker. The false alarm rate was monitored and thresholds were retested if the rate was too high. The animals learned to respond reliably to a suprathreshold tone in an average of 20 sessions consisting of 10-20 trials per session.

Although a complete audiogram was successfully obtained using this procedure, there was considerable variability in the animals' thresholds. One factor contributing to this was the variation in the intensity of the sound reaching an animal, which depended both on where the animal was standing and whether its head was oriented toward or away from the loudspeaker. Another factor was the lack of an effective observing or ready response and an animal would 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 trained with this procedure, especially when the task involves discriminating between two different sounds rather than simply detecting a sound (Ehret, 1975b; 1976b).

Because an animal's head is not fixed, this procedure cannot be used for sound-localization testing. However, this could be corrected by adding a second water spout to serve as an observing response. For example, an animal could be trained to place its mouth on one water spout while waiting for a tone and to contact a second water spout located directly below it when a tone is presented (a similar procedure has been used with other species; e.g., Heffner and Heffner, 1983). This modification would both fix an animal's head within the sound field and allow for the automated presentation of trials and the recording of responses. In addition, the inclusion of an observing response might increase the success rate, although the amount of time needed to train an animal would increase.

Recently, a modification of the procedure used by Birch and her colleagues has appeared which contains several new features (Prosen et al., 2000). Photocells are used to determine the location of an animal and trials start automatically once it has entered the listening side of the test cage. Instead of requiring the animal to press a lever when it hears a tone, a response is counted as soon the animal crosses over to the side of the cage containing the water reward. Errors, both false alarms and failure to respond to a tone (misses) are punished by a time out which requires 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.

2.4. Avoidance conditioning

Two procedures have made use of avoidance conditioning to obtain auditory thresholds in mice. The first procedure used a shuttle box, also known as a double grill box, to obtain absolute thresholds (Schleidt and Kickert-Magg, 1979). This procedure consists of placing an animal in a test cage consisting of two compartments (the shuttle box). The animal is then required to cross from one compartment to the other whenever it hears a tone in order to avoid an electric shock applied to the bars of the cage floor. This task is basically a go/no-go procedure in which the animal makes a response to avoid shock, as opposed to obtaining a positive reward as in the go/no-go procedures described above (section 2.2).

The second procedure trained mice to jump onto a platform to avoid electric shock in response to a change in the frequency of a tone (Kulig and Willott, 1984). The animals were placed in a box with a grid floor and trained to jump onto a small platform whenever an ongoing train of tone pips of the same frequency was replaced by a train of tone pips that alternated in frequency. The animals were given 5 s to respond to the change in frequency after which electric shock was delivered through the grid floor. Jumping onto the platform when the tone pips were all the same frequency (false alarms) was punished by allowing the platform to collapse.

Although the shock avoidance procedure has an advantage in that it is not necessary to deprive an animal of food or water, it does not appear to work well with mice. The animals tested in the shuttle box required 3 months of training and the investigators were only able to train 6 out of the 15 animals. Furthermore, the animal's head is not fixed in the sound field and animals may continuously cross back and forth between the two compartments to avoid the shock thus rendering them untestable. Similar problems were encountered in the test in which the animals jumped onto a platform to avoid shock. Although only 1 of the 12 animals failed to reach criterion, the false positive rates were high (usually exceeding 20%) and performances even at large frequencies differences tended to be poor. As better procedures are now available for testing mice, there is no longer any reason to consider using shock avoidance.

2.5 Approach the source of a sound

An attempt was made to assess the ability of mice to localize sound by training them to approach the source of a sound (Ehret and Dreyer, 1984). In this procedure, a mouse was placed in a circular test cage and trained to initiate trials by contacting a water spout located in the center of the cage, which turned on a sound from one of three loudspeakers placed at the edge of the test 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 animal's head is precisely oriented with respect to the loudspeakers by requiring the animal to stand on a platform to reach the center spout. A contact switch then detects when then animal is in the proper position and turns on the sound (Thompson et al., 1974). One possible explanation for the inability of mice to do well in this task is that small prey animals may have a general reluctance to approach the source of a sound, especially if they have to cross a large open area. If so, then mice might perform better in a smaller test cage than the 1.55 m-diameter cage used by Ehret and Dreyer (1984).

It should be noted that sound localization ability can also be assessed using the conditioned suppression/avoidance procedure in which an animal ceases to drink when a sound comes from one direction, but not from another (Heffner et al., in press). Indeed, the two procedures, approaching the source of a sound and suppressing to a change in the location of a sound source, have been shown in other mammals to give similar results (Heffner and Heffner, 1988a, 1992). However, the suppression technique, which allows an animal to respond immediately, may be a more accurate measure of sensory ability because an animal that is required to approach the source of a sound may become distracted before it can complete its response.

2.6 Galvanic skin response (GSR) audiometry

The galvanic skin response (GSR) is a measure of skin conductance and is a popular measure of autonomic arousal (e.g., Woodworth and Schlosberg, 1965). It occurs as an *un*conditioned response to a sudden loud which habituates with repeated presentation. However, a GSR can be obtained to low-intensity sounds by pairing the sounds with electric shock. In this way the GSR has been used to obtain absolute thresholds in mice (Berlin, 1963).

For testing, a mouse is sedated to reduce extraneous movements and to allow it to be restrained. Shock electrodes are attached to the animal's front paws and recording electrodes to its hind paws. For conditioning, 1-s tones are presented at random intervals accompanied by 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 investigator, although GSR conditioning may be of interest in its own right, it does not appear to be a good method for assessing auditory sensitivity in individual animals (Berlin, 1963).

2.6 Summary of procedures using conditioned responses

Of the five behavioral conditioning procedures reviewed here, the conditioned suppression/avoidance procedure appears to be the most effective for the following reasons: it has the shortest training time, it allows the most trials to be given, it holds the animal's head fixed in the sound field thus permitting accurate specification of the auditory stimulus reaching the animal, and it works with animals considered difficult to test. Although go/no-go and eyeblink procedures can also give good results, these two procedures do not fix an animal's head within the sound field and will not work with all animals, especially those that are overly active.

3. Unconditioned response procedures

The simplest methods for assessing hearing in mammals takes advantage of an animal's unconditioned responses to sudden loud sounds. As a result, it is possible to show that an animal can detect a sound without having to engage in lengthy training. In most cases, such tests are limited in that animals show an unconditioned response only to sounds that are very loud, the response habituates, and the procedures cannot be used to measure an animal's ability to 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 rise/fall time). Although habituation of the startle reflex does occur, it is generally possible to obtain a large number of trials (e.g., 60) within a single session.

Because the startle reflex occurs only to relatively loud sounds, it cannot be used to determine absolute sensitivity. However, it can provide information that may be used to supplement threshold measurements (e.g., Parham and Willott, 1988). For example, a normal startle to loud sounds in an animal with a hearing loss could indicate the occurrence of recruitment, a phenomenon in which absolute thresholds are elevated, but the apparent loudness of sounds at suprathreshold levels is unchanged (e.g., Moore, 1997). Thus, the acoustic startle reflex can provide additional information about an animal's hearing ability.

3.2 Prepulse inhibition

Although the acoustic startle reflex itself can only determine an animal's ability to respond to loud sounds, the latency or amplitude of the response can be modified by a less intense sound that precedes the startle sound, but which does not itself cause a startle (e.g., Hoffman and Ison, 1980). Thus, the ability of an animal to detect a particular sound can be investigated by determining if presenting that sound before the startle sound modifies the resulting reflex, a procedure referred to as reflex modification or prepulse inhibition (see Ison, in press).

As in the startle reflex test described above, a mouse is placed in a cage that has an accelerometer attached to it to detect the animal's movements (e.g., Ison et al., 1998; Willott and Turner, 1999). A startle sound, such as a 25-ms noise burst at 115 dB with a near instantaneous rise/fall time, is presented at random intervals when the animal is sitting quietly. The startle stimulus is preceded on most trials (e.g., 75%) by a prepulse stimulus, such as a 40-ms tone, with the startle stimulus presented alone on the remaining trials for comparison. A typical interval between the two stimuli is 100 ms, although the optimal interval must be determined empirically. The effect of the prepulse stimulus is expressed as a percent reduction of the unmodified startle response. Test sessions may last 1 hr during which about 50-100 trials are presented.

Prepulse inihbition can be used to measure both absolute thresholds and the ability to discriminate between different sounds. Absolute thresholds are determined by reducing the intensity of the prepulse stimulus until it no longer has an effect on the startle reflex. The ability to discriminate stimuli is determined by using a change in an ongoing sound as the prepulse stimulus. For example, the ability to discriminate continuous noise from noise containing a gap is determined by presenting ongoing noise interrupted by a gap that occurs just before the presentation of the startle stimulus, i.e., the gap served as the prepulse stimulus. This procedure has produced reasonable gap detection thresholds in mice (Ison et al., 1998) and there appears to be no reason that it cannot be used for other auditory discriminations. That is, frequency- and intensity-difference thresholds, as well as sound-localization thresholds, could be determined by using a prepulse stimulus that consists of a change in the frequency, intensity, or location of an ongoing sound.

Although prepulse inhibition appears to be ideal for sensory testing, the question arises as to whether it is as sensitive as conditioned response procedures. First, experience with conditioning procedures suggests that animals must learn to listen before they becomes reliable observers, i.e., initial thresholds are generally higher than those obtained in later sessions (e.g., Stebbins, 1970). Because prepulse inhibition does not involve any training for vigilance, it might not reflect an animal's best sensitivity. Second, the current method for fixing an animal's head in the sound field involves tranquilizing the animal and holding its head in place with a wooden Q-tip glued to the skin of its head (e.g., Ison and Agrawal, 1998), raising the question of whether sedation would affect thresholds.

At present, there is reason to believe that prepulse inhibition can yield sensitive thresholds. Specifically, a reflex modification study using rats obtained thresholds as low as 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 animals in the same acoustic environment using a conditioned response procedure. Moreover, the entire audiogram should be determined to ensure that the procedure is equally sensitive at all frequencies as at least one procedure, the conditioned GSR, appears to underestimate high frequency hearing (see section 2.5).

In summary, prepulse inhibition appears to be a simple and rapid method for assessing hearing. Because it uses a natural reflex, an animal requires no training and useable results may be obtained in the first session. However, it should be noted that tests are sometimes carried out with 2-4 days between sessions to avoid habituation, with the result that some tests require as much as two months to conduct (e.g., Ison and Agrawal, 1998). Moreover, the optimal parameters may vary with the particular task making it necessary to conduct pilot studies before detailed testing can begin. For example, it is helpful to know the particular startle stimulus and interval between the prepulse and startle stimuli that yields the best results, as well as the maximum number of trials that can be given without causing excessive habituation.

3.3 Pinna movements

Because mammals with mobile ears will move their pinnae when presented with an unexpected sound, one of the first tests of animal hearing was to look for pinna movements in response to sound. One type of pinna movement is the Preyer reflex, which is a movement of the pinna in response to loud sounds (Ehret, 1983; Francis, 1979). This response is considered to be a startle reflex as it occurs only to loud sounds and thresholds obtained with it are about 60-90 dB above those obtained with conditioning procedures (e.g., Hack, 1968; Markl and Ehret, 1973).

Another type of response is the pinna movements that occur in reaction to sounds as low as 25 dB (re 20 μPa), much lower than the sounds that elicit a Preyer reflex (Ehret, 1976a; 1983). In this case, the animal appears to be making use of the directional filtering properties of its pinnae to search its auditory environment. Although the response rapidly habituates, it can be reinstated by briefly handling the animals, which probably has the effect of sensitizing them (Ehret, 1976a).

Although both of these responses are of interest in their own right, the Preyer reflex occurs only to very loud sound and it is unknown whether pinna movements to less intense 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 relative response of mice to tones from 2-40 kHz delivered at a constant sound pressure level of 100 dB (Berlin et al., 1968). As in the conditioned GSR procedure, the mouse is sedated to reduce extraneous movements and GSR recording electrodes attached to its hind feet. The magnitude of the GSR appears to parallel its audiogram, suggesting that the unconditioned GSR might provide an equal loudness contour. However, given the uncertainty as to whether the GSR is an accurate indicator of high-frequency hearing (see section 2.5), other estimates of loudness, such as the acoustic startle reflex, might provide more accurate results.

3.6 *Summary of unconditioned response procedures*

Of the five unconditioned procedures reviewed here, prepulse inhibition appears to hold 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 as those obtained with procedures using conditioned responses. In addition, the acoustic startle reflex itself is a useful indicator of an animal's responsiveness to loud sounds and is therefore an important adjunct to tests of absolute threshold. Both prepulse inhibition and the acoustic startle reflex have been the subject of numerous studies, with the result that much is known about them (e.g., Ison, in press). Moreover, the acoustic startle response is measured objectively whereas most other unconditioned procedures rely on the subjective report of the experimenter. Therefore, these two procedures are to be preferred when assessing hearing with unconditioned procedures.

4. Conclusion

Because mice play an key role in the study of the genetics of hearing, it is important to develop procedures that can quickly and accurately measure their hearing. Not only should a procedure be capable of assessing absolute sensitivity, but it should be able to determine an animal's ability to discriminate between sounds as well.

Of the procedures reviewed here, two hold the most promise for testing mice. The first, conditioned suppression/avoidance, is a relatively simple procedure as the animals need only stop drinking when they hear a sound that signals impending shock. As a result, it is possible to train animals in a minimum amount of time. In addition, requiring an animal to drink from a water spout fixes its head within a sound field, which allows for precise measurement of the sound reaching the animal and makes it possible to test sound localization. Indeed, this is one of the few procedures that can be used to test any auditory discrimination.

However, the current conditioned suppression/avoidance procedure could be improved by increasing the speed with which thresholds are assessed. For example, current practice for assessing absolute thresholds is to present tones of a particular intensity in blocks of 5 or more trials, calculate the animal's performance, and then change to a new intensity. Because mice require little water and thus work for only 15-20 min, only one threshold can be obtained per daily session using this procedure. However, the speed with which thresholds are determined could be increased by using an automated tracking procedure in which the intensity of the tone is changed from trial to trial, as originally recommended by Sidman and his colleagues (1966), instead of collecting information in blocks of trials. Other changes that might also increase the 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 as the animals need not be trained. However, it remains to be determined whether absolute thresholds obtained with this procedure are as sensitive as those obtained with conditioned response procedures. In addition, it is important to find a good way to keep an animal's head fixed within the sound field as there is the possibility that the current technique, which involves sedation, might affect thresholds. Should prepulse inhibition prove to provide valid thresholds, it could easily supplant conditioned response procedures for assessing hearing in mice and other animals. In spite of the fact that it may be necessary to space test sessions several days apart to reduce habituation, prepulse inhibition is simpler to use and would be able to test more animals in the same amount of time than any of the conditioned response procedures, including conditioned suppression/avoidance.

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