Behavioral audiograms of homozygous med^{J} mutant mice with sodium channel de¢ciency and una¡ected controls

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Complete behavioral audiograms were determined for med^{J} mice (F1 offspring of C57BL/6J×C3HeB/FeJ) and unaffected controls from the same F1 background. The med^{J} mutation results in greatly reduced levels of Scn8a voltage-gated sodium channels, which causes abnormal conduction of action potentials throughout the nervous system and may account for the virtual absence of spontaneous bursting activity in the dorsal cochlear nucleus. The med^{J} mice also have tremors, display dystonic postures, and drag their hind legs. The mice were tested using a conditioned suppression/avoidance procedure, with minor modifications of the apparatus made to accommodate the motor-impaired med^{J} mice. Thresholds were repeatedly obtained up to the age of 50 weeks to determine if the animals developed a hearing loss with age. The results indicate that med^{J} mice have normal thresholds, with the first signs of hearing loss (detectable at 80 kHz) appearing for both the med^{J} and normal mice by 48 weeks. Neither the med^{J} nor the normal mice could hear below 1 kHz, indicating that house mice fall into the group of mammals with poor low-frequency hearing. The results also demonstrate that the conditioned suppression/avoidance procedure is well suited for assessing hearing in severely impaired, as well as normal, mice and that it can provide for the rapid determination of thresholds necessary to follow changes in hearing that may occur as the result of age, disease, mutation, or drugs. □ 2002 Published by Elsevier Science B.V.

Audiogram; Sodium channel; Med^{J}; Mutation; Behavioral method; Test reliability

1. In rod c ion

The existence of numerous mouse strains with hearing impairments has made mice an important tool for studying the genetics of hearing (e.g., Henry and McGinn, 1992; Probst and Camper, 1999; Steel, 1995

that are well above its absolute threshold and has proven unreliable with mutant mice (Cheatham et al., 2001). Another procedure is re£ex modi¢cation, also referred to as prepulse inhibition, in which the ability to detect and discriminate a low-intensity sound is judged by its inhibitory e¡ect on the startle response to a more intense sound (e.g., Carlson and Willott, 2001; Ison, 2001). Although important in its own right, the prepulse inhibition response is also a test of motor re£exes and sensory gating. Thus, di¡erences in the performances of di¡erent strains of mice could be due to nonauditory factors and thus not re£ect true auditory sensitivity (Paylor and Crawley, 1997).

Although conditioning procedures are commonly used to obtain auditory thresholds in mammals, they are not often used with mice because small animals are considered di/cult to train (Prosen et al., 2000). However, past research has shown that mice are far from intractable. Indeed, not only have complete audiograms been obtained for both domestic and wild-caught house mice (Ehret, 1974; He¡ner and Masterton, 1980), but their frequency- and intensity-di¡erence limens, temporal integration, masking, and sound localization ability have also been determined (Ehret, 1983; He¡ner et al., 2001b).

The primary purpose of this study was to determine the absolute thresholds of mutant mice suspected of having abnormal auditory function. Homozygous med^J mice have greatly reduced levels of Scn8a voltage-gated sodium channels (Kohrman et al., 1996; Sprunger et al., 1999) that are normally found not only at the nodes of Ranvier, but also at synapses and on dendrites (Caldwell et al., 2000). One of the consequences of the med^J mutation is greatly slowed neural conduction (Kohrman et al., 1996; Meisler et al., 2001) and a failure of the Purkinje cells in the cerebellum to show normal spontaneous activity (Raman and Bean, 1999); this latter ¢nding may partly account for their movement disorders as these animals have tremors, display dystonic postures, and drag their hind legs. Although their auditory system has not been well studied, it is known that med^J mice do not show the typical bursting spontaneous activity that is seen in cartwheel cells in dorsal cochlear nucleus (Chen et al., 1999). Thus, one might reasonably expect some aspect of their hearing to be abnormal.

This study also provided the opportunity to ask two additional questions. The ¢rst was whether the conditioned suppression/avoidance procedure could be used to assess hearing in mice with severe motor disorders. The second was whether the domestic house mouse is able to hear frequencies below 1 kHz, a question that has recently taken on theoretical signi¢cance (He¡ner et al., 2001a).

2. Me hod

Behavioral audiograms were obtained for seven mice. Three mice (animals A, B, and C) were homozygous for the recessive med^J mutation (which results in a reduced

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by the animal's head and pointing it directly at the loudspeaker (0‡ incidence). Care was taken to produce an homogeneous sound ¢eld (within □1 dB) in the area occupied by the animal's head and ears when it was in contact with the spout.

A thirsty mouse was ¢rst trained to maintain steady contact with the reward spout in order to receive a slow but steady trickle of fruit juice, delivered at a rate of 7 ml/h. Because the constant tremors of the medJ mice prevented them from making uninterrupted contact with the reward spout, pauses of 100 ms or less were ignored for all mice. A train of four tone pulses (400 ms on, 100 ms o¡) was then presented at random intervals and followed at its o¡set by a mild electric shock (300

frequency that previous studies had indicated is in the region of best hearing for house mice (e.g., Berlin, 1963; He¡ner and Masterton, 1980; Ehret, 1974). The normal mice required from three to six conditioning sessions before their lowest threshold was reached. Thresholds at this and other frequencies were obtained with a minimum of three sessions per frequency, with the stipulation that thresholds from at least two sessions be within 3 dB of each other. On average, only one in four thresholds required more than three test sessions to meet this criterion.

The med^{J} mice were accustomed to drinking for nine sessions, during which time the problem of their intermittent contact with the reward spout, caused by their tremor, was solved by disregarding contact breaks of $\leq$ 100 ms. The animals were then trained to stop drinking when a 16 kHz tone was presented. After 12 sessions it was realized that their tremor also prevented them from reliably receiving the shock through the reward spout, a problem that was solved by shocking their feet through a £oor grid. Subsequently, these mice required two to seven conditioning sessions before their lowest threshold was reached. Once trained, the med^{J} mice took no longer to test than the normal mice.

The performance of all seven mice at suprathreshold intensities was good, with hit rates of 85 to 100% and false alarm rates of 0 to 10%. False alarm rates remained low and stable, rising only when the animals were tested at intensities that proved to be below their thresholds. The psychophysical functions of the med^{J} mice did not appear to di¡er from those of the normal mice as both showed good suprathreshold performance with relatively sharp slopes (

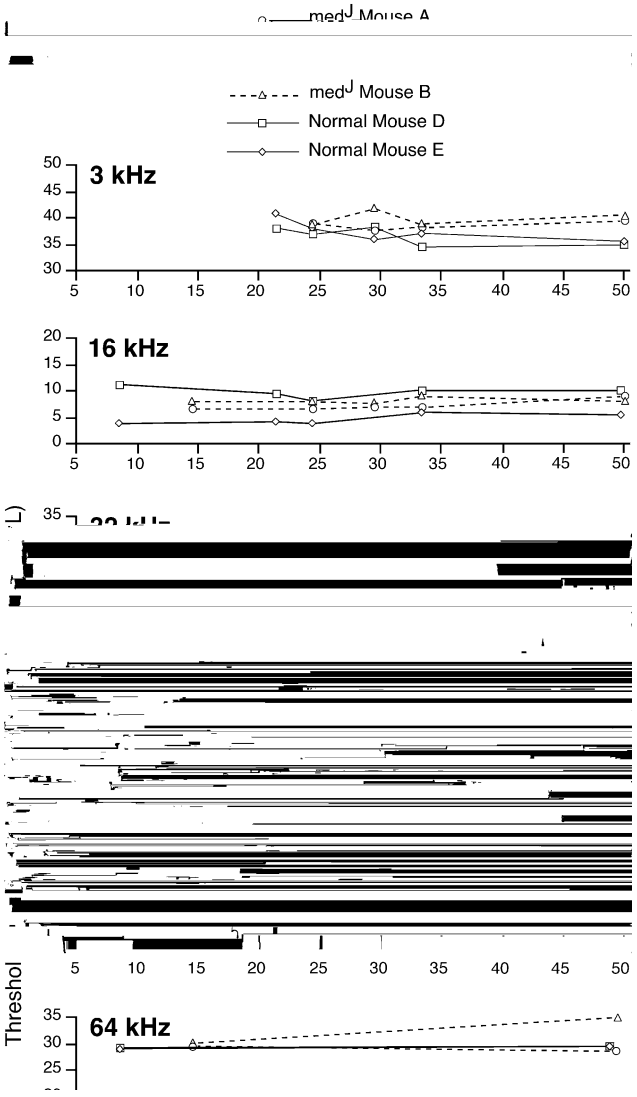

Fig. 4. Thresholds for four frequencies were retested up to 50 weeks

from at least two sessions. As can be seen, there was no obvious hearing loss up to 34 weeks at any of these frequencies. Indeed, thresholds were quite stable, varying by as little as 0.5 dB (Mouse A at16 and 32 kHz) to 5 dB (Mouse D at 3 kHz). The fact that the thresholds show little change also demonstrates the test^retest reliability of the behavioral procedure.

The only hearing loss detected was at 80 kHz, the highest frequency retested. At 48 weeks of age, the thresholds for the normal mice had increased by 10 dB whereas those of the med^J mice had increased by 23 and 27 dB (mouse A and B, respectively). To determine if the hearing loss extended below 80 kHz, all four mice were then tested at 64 kHz In contrast to the obvious loss of sensitivity at 80 kHz, thresholds at 64 kHz were within 5 dB of those obtained at 8^14 weeks of age. Thus, these mice show a high-frequency hearing loss at 80 kHz that appears after 34 weeks of age with the med^J mice showing a greater loss than their normal littermates.

4. Di c ion

The conditioned suppression/avoidance procedure was chosen for assessing hearing in these mice because it requires very little learning and very little motor prowess on the part of an animal. To perform the task, an animal need only drink from a spout and then momentarily withdraw when a warning signal indicates impending shock ^ a response not unlike the natural reaction of an animal to signs of danger. Indeed, this task has been used successfully to assess hearing in severely brain-damaged and in otherwise intractable animals including wild-caught mice (e.g., He¡ner and He¡ner, 1995; He¡ner and Masterton, 1980; He¡ner et al., 2001a). Furthermore, requiring an animal to place its mouth on a reward spout ¢xes its head in the sound ¢eld making it possible to accurately specify the sound reaching the ears.

Only minor modi¢cations of the apparatus were necessary to accommodate the med^J mice. Because their tremors prevented them from maintaining steady contact with the spout, it was necessary to record only those breaks in contact that exceeded 100 ms. In addition, their intermittent contact also made it necessary to deliver the shock through a foot grid so that it would be consistent from trial to trial. Finally, initial training indicated that, unlike the normal mice, the med^J mice would not take su/cient water in a single session to maintain their body weight. This problem was solved by using fruit juice as a reward, a technique that also increased the intake of the normal mice allowing more trials to be given per session. Indeed, the only di¡erence in testing mice, as opposed to larger animals, is that their low £uid intake limits the number of trials that can be given in a session.

In summary, the conditioned suppression/avoidance task provides a useful procedure for testing the hearing of normal mice as well as those with severe motor disorders. The response is easily learned by the animals and thresholds can be obtained daily making it possible to follow changes in hearing that might occur as the result of age, disease, noise exposure, or drugs.

Neurons in med^J mice are de¢cient in Scn8a voltage-gated sodium channels (Kohrman et al., 1996), channels

which play an important role in the production of action potentials (Llinas, 1988). As a ¢rst step in determining the e¡ect of this mutation on hearing, the dorsal cochlear nucleus of med^{J} mice was studied in brain slices (Chen et al., 1999). The results of that study revealed that neurons in the dorsal cochlear nucleus of these animals do not show the typical bursting spontaneous activity that is found in normal mice, although they do show regular spontaneous ¢ring. This ¢nding suggested that some aspect of their hearing might be abnormal. The results of the present study indicate that the med^{J} mouse has normal sensitivity, at least for pure tones of 400-ms duration. Whether the animals might have di/culty detecting shorter-duration sounds or whether their ability to discriminate the frequency, intensity, or locus of sounds is impaired remains to be determined.

The audiogram obtained by Ehret on NMRI mice has long served as the standard behavioral audiogram for domestic house mice (e.g., Ehret, 1974). As can be seen in Fig. 5, the present audiogram agrees closely with that audiogram at all frequencies except one. Whereas Ehret obtained a threshold of 36 dB at 1 kHz, we found the 1-kHz threshold to be at least 92 dB. The most likely explanation of the di¡erence is that the 1-kHz signal used by Ehret contained overtones to which the animals were responding. Indeed, as we found, it can be di/cult to generate low frequencies at high intensities without producing overtones to which the mice are sensitive.

The relative insensitivity of house mice to frequencies below 2 kHz is supported by the fact that no other behavioral study has found good low-frequency hearing in mice. In studies using CBA/J mice, Berlin (1963) obtained a 1-kHz threshold of 91 dB and Birch et al. (1968) obtained a 1-kHz threshold of 70 dB. Using albino house mice, Schleidt and Kickert-Magg (1979) obtained a 1-kHz threshold of 90 dB. Finally, a study of wild-caught house mice found that their 2-kHz threshold was 70 dB (lower frequencies not being tested, He¡ner and Masterton, 1980; see Fig. 5). Indeed, the limited low-frequency hearing of wild-caught mice indicates that our failure to replicate the 1 kHz obtained by Ehret is not due to strain di¡erences. That is, strain di¡erences in hearing are the result of muta-

the middle of the audiogram, from 4 to 32 kHz. This di¡erence suggests that perhaps domestic mice have lost some of their midrange sensitivity with no e¡ect on their high- and low-frequency hearing abilities. However, it would be appropriate to verify this ¢nding by testing wild and domestic mice in the same apparatus using the same stimulus-generation and measuring equipment before attributing much signi¢cance to this di¡erence.

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