

## Behavioral audiograms of homozygous *med<sup>J</sup>* mutant mice with sodium channel deficiency and unaffected controls

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### Abstract

Complete behavioral audiograms were determined for *med<sup>J</sup>* mice (F1 offspring of C57BL/6J × C3HeB/FeJ) and unaffected controls from the same F1 background. The *med<sup>J</sup>* 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<sup>J</sup>* 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<sup>J</sup>* 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<sup>J</sup>* mice have normal thresholds, with the first signs of hearing loss (detectable at 80 kHz) appearing for both the *med<sup>J</sup>* and normal mice by 48 weeks. Neither the *med<sup>J</sup>* 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.

*Key words:* Audiogram; Sodium channel; *Med<sup>J</sup>*; Mutation; Behavioral method; Test reliability

### 1. Introduction

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). To take full advantage of this opportunity, it is necessary to determine the nature and degree of hearing impairments in the various strains. To date, the

primary means for assessing hearing in mice have been physiological, specifically, the auditory brainstem response and evoked otoacoustic emissions (e.g., Parham et al., 2001; Zheng et al., 1999). These tools are useful in that they provide a rapid means for assessing the integrity of the ear and auditory brainstem. Ultimately, however, it is necessary to determine an animal's hearing behaviorally, for not only does behavior reflect an animal's actual hearing ability, but it is the only means for assessing the ability to discriminate such features as frequency, intensity, and location – abilities for which there are no valid physiological measures.

One approach has been to use unconditioned response procedures to obtain behavioral measures of hearing (for reviews see Ehret, 1983; Heffner and Heffner, 2001). The simplest such response is the acoustic startle, which is easily evoked. However, this procedure can only demonstrate an animal's ability to hear sounds

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*Abbreviations:* SPL, sound pressure level re 20  $\mu$ Newton/m<sup>2</sup>

that are well above its absolute threshold and has proven unreliable with mutant mice (Cheatham et al., 2001). Another procedure is reflex modification, also referred to as prepulse inhibition, in which the ability to detect and discriminate a low-intensity sound is judged by its inhibitory effect 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 reflexes and sensory gating. Thus, differences in the performances of different strains of mice could be due to non-auditory factors and thus not reflect 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 difficult 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; Heffner and Masterton, 1980), but their frequency- and intensity-difference limens, temporal integration, masking, and sound localization ability have also been determined (Ehret, 1983; Heffner 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 finding 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 first 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 significance (Heffner et al., 2001a).

## 2. Methods

### 2.1. Subjects

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 number of Scn8a sodium channels) and are hereafter referred to as  $med^J$ . Their hearing was compared to that of four phenotypically unaffected mice (animals D, E, F, and G) of the same F1 genetic background, hereafter referred to as normals.

The Scn8a sodium channel is one of the four major voltage-gated sodium channels in the nervous system (Plummer and Meisler, 1999). It is found at pre- and postsynaptic receptor sites as well as at the nodes of Ranvier, where it appears to be the only sodium channel (Caldwell et al., 2000). Complete absence of these channels results in paralysis and juvenile death (Burgess et al., 1995). However, when maintained on a genetic background containing the resistant allele *Scnm* at the master locus (Sprunger et al., 1999), the  $med^J$  mutation is accompanied by the survival of a small percentage of Scn8a voltage-gated sodium channels that enables the mice to survive past one year of age, although the conduction of action potentials is severely compromised (Kohrman et al., 1996; Meisler et al., 2001). These mice have motor abnormalities that include tremors, weak hind limbs (they drag their hind legs), and abnormal (dystonic) postures (Sprunger et al., 1999).

The F1  $med^J$  mice used here, and the phenotypically normal mice, were produced by crossing two congenic lines, C57BL/6J- $med^J/+$  and C3HeB/FeJ- $med^J/+$ . They had one copy of the resistant *Scnm* allele, which they received from the C3HeB/FeJ parent (L. Sprunger and M. Meisler, personal communication). Thus, the  $med^J$  mice were  $med^J/med^J$ , while the phenotypically normal mice were either heterozygous for the  $med^J$  mutation ( $med^J/+$ ) or homozygous wild types ( $+/+$ ). All of the mice, then, were from the same homogeneous F1 background and differed only with respect to the  $med^J$  mutation.

Four mice were retested up to the age of 50 weeks. The  $med^J$  mice were approximately 13 weeks old, weighing 18 g at the beginning of training and 21–24 g at the end of testing. The normal mice were approximately seven weeks old, weighing 22.5–23.5 g at the beginning of training and 27–28 g at the end of testing.

The mice were housed in solid bottom cages (28×18×13 cm) and maintained on standard rodent diet (Harlan Teklad), supplemented with slices of apple. No animals died and all gained weight during testing – we attribute this to the use of fruit juice as a reward during testing and to apple supplements when the animals were not on test. All procedures were approved by

the Animal Care and Use Committee of the University of Toledo.

## 2.2. Behavioral apparatus

Testing was conducted in a carpeted, double-walled acoustic chamber (IAC model 1204; Industrial Acoustics Co., Bronx, NY, USA;  $2.55 \times 2.75 \times 2.05$  m), the walls and ceiling of which were lined with acoustic foam. The equipment for stimulus generation and behavioral measurement was located outside the chamber.

The animals were tested in a cage ( $14 \times 8 \times 10$  cm) constructed of 0.5-in (1.27-cm) hardware cloth, and mounted on a tripod 1 m above the floor (Fig. 1). A reward spout (2-mm-diameter brass tube, topped with a  $5 \times 8$ -mm lick plate) was mounted vertically so that it projected up through the cage bottom with the top approximately 2 cm above the cage floor. Fruit juice (a cantaloupe and pear juice mix) was used as a reward instead of water because the animals took more of it, allowing for longer test sessions. The juice also helped the med<sup>J</sup> mice maintain a healthy body weight. The reward was dispensed from a 3-ml syringe using a commercial syringe pump (Yale Apparatus, YA-12) connected to the reward spout via plastic tubing. To eliminate the noise generated when the syringe pump was activated, the syringe pump was housed in a wooden box ( $40 \times 30 \times 28$  cm), lined with eggcrate foam, and placed on the floor of the sound chamber behind the test cage.

A contact circuit, connected between the reward spout and cage floor, served to detect when an animal made contact with the spout and to activate the syringe pump. Requiring an animal to keep its mouth on the reward spout served to fix its head in the sound field, allowing for precise measurement of the intensity of the sound at its ears. A shoulder-high fence was placed in front of the cage to ensure that the animal was facing directly forward when licking the spout. Initially, a 2-mm-thick dampened sponge was placed on the floor of the cage on which the animal stood, and electric shock was delivered between the reward spout and cage floor. However, because of their tremors, the med<sup>J</sup> mice made intermittent contact with the spout and thus did not reliably receive the brief (300 ms) shock. As a result, it was necessary to use a grid floor so that all mice could be shocked through their feet. The grid consisted of 22-gauge copper wire inserted onto a  $4.5 \times 4$  cm piece of perforated circuit board, at 0.1-in (2.54-mm) intervals. Electric shock was delivered between alternate wires of the grid floor as well as between the reward spout and cage floor. A 25-watt light, mounted 0.5 m below the cage, was turned on and off simultaneously with the shock and the animals learned not to return to the spout until the light was off.

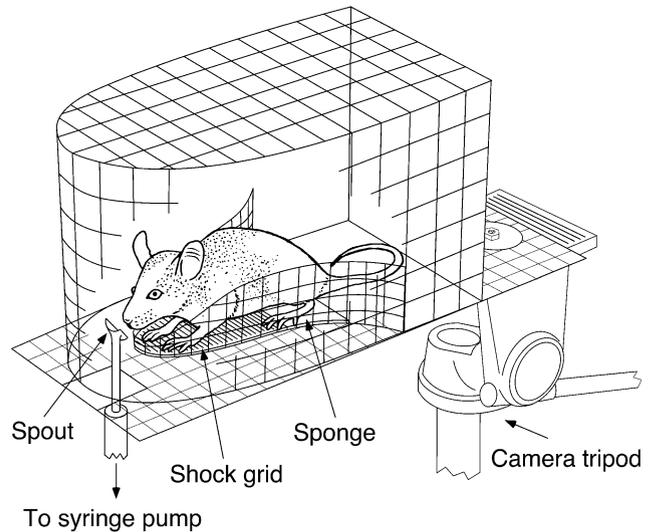


Fig. 1. Cage for testing mice. An animal drank from the reward spout while standing on a wire grid floor. A shoulder-high fence helped position the animal directly in front of the loudspeaker (not shown.) The animal's feet were moistened by a dampened sponge to ensure electrical contact with the floor.

## 2.3. Acoustic apparatus

Sine waves from 1 to 90 kHz were generated using a digital signal generator (Stanford Research Systems SR 770), attenuated (Coulbourn S85-08), pulsed (400 ms on and 100 ms off for four pulses), and gated by a rise/fall gate (Coulbourn S84-04, cosine gating) with rise-decay times of 20 ms for the 1 kHz tone and 10 ms for frequencies of 1.4 kHz and higher. The signal was then band-pass filtered (Krohn-Hite 3202,  $\pm 1/3$  octave settings, 24 dB/oct rolloff), amplified (Crown D75), and sent to a loudspeaker. The electrical signal was monitored at the amplifier output for distortion and noise with an oscilloscope (Tektronix TDS 210). The loudspeaker was located approximately 1 m in front of, and level with, an animal's head when it was drinking from the spout. A 6-in (15.2-cm) loudspeaker (Infinity RS2000) or a 12-in (30.5-cm) woofer was used for low frequencies (1, 1.4, and 2 kHz), and a leaf tweeter (Panasonic EAS10TH100A) or piezoelectric speaker (Motorola KSN 1005A) was used for higher frequencies (3, 4, 8, 16, 32, 50, 64, 80, and 90 kHz).

The acoustic signal was analyzed for overtones and the sound pressure level (SPL re  $20 \mu\text{Newton/m}^2$ ) was measured using a 1/4-in (0.64-cm) Brüel and Kjaer (B and K) microphone (model 4939, reading corrected for the protection grid), preamplifier (B and K 2619), conditioning amplifier (B and K Nexus 2690) and spectrum analyzer (Stanford Research Systems SR 770). The measuring system was calibrated with a piston-phone (B and K 4230). Sound measurements were taken by placing the microphone in the position occupied

by the animal's head and pointing it directly at the loudspeaker ( $0^\circ$  incidence). Care was taken to produce an homogeneous sound field (within  $\pm 1$  dB) in the area occupied by the animal's head and ears when it was in contact with the spout.

#### 2.4. Behavioral procedure

A thirsty mouse was first 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 med<sup>J</sup> 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 off) was then presented at random intervals and followed at its offset by a mild electric shock (300 ms maximum duration,  $\leq 1.25$  mA) delivered between the spout and cage floor, as well as between alternate bars of the floor. If the mouse was not in contact with the spout during the last 200 ms of a tone trial, the shock was not given, although the shock light was briefly illuminated to provide feedback for a successful avoidance. Thus, the mouse learned to avoid the shock by breaking contact with the spout whenever it detected a tone. The shock was adjusted for each mouse to the lowest level that would reliably produce an avoidance response. The shock level was considered mild because the mice did not develop a fear of the spout and readily returned to it after the shock had been delivered.

Test sessions were divided into 2-s trials, separated by 0.5-s intertrial intervals, with a typical session lasting 20–30 min. A tone was presented during approximately 22% of the trials (warning trials) whereas the remaining trials consisted of silence (safe trials). The contact circuit was used to detect whether an animal was in contact with the spout during the last 200 ms of every trial. If an animal broke contact for more than half of the 200-ms response period, a detection response was recorded. This response was classified as a hit if a tone had been presented, or as a false alarm if no tone had been presented. Thresholds were initially estimated using the method of descending limits, in which the intensity of a tone was reduced in 10-dB steps until threshold was approached, at which point six to eight warnings (and associated safe trials) were given at each intensity. Near threshold, the intensity was decreased in 5-dB steps until the animal could no longer detect the tone above chance levels (i.e., the hit and false alarm rates did not differ significantly;  $P > .01$ , binomial distribution). Hit and false alarm rates were determined for each block of trials at each stimulus intensity. The hit rate was then corrected for false alarms to produce a performance measure (Heffner and Heffner, 1995) according to the formula: Performance = Hit rate – (False

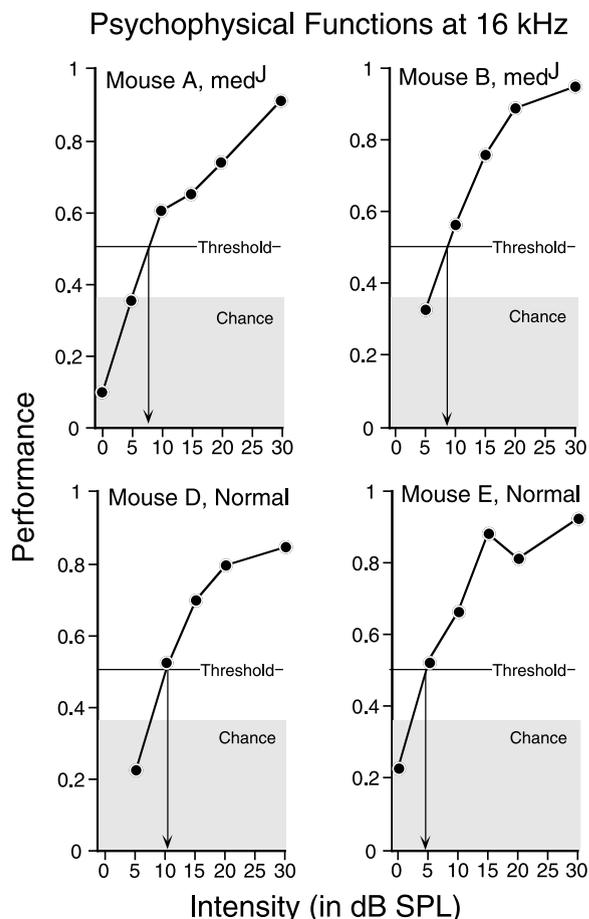


Fig. 2. Psychophysical functions of four mice at 16 kHz. The med<sup>J</sup> mice did not differ from the normal mice either in asymptotic performance or in steepness of their psychophysical functions. Thresholds at this frequency ranged from 4 to 11 dB with a mean of 7 dB. Arrows indicate the 50% threshold.

alarm rate  $\times$  Hit rate). This measure proportionately reduces the hit rate by the false alarm rate and varies from 0 (no hits) to 1 (100% hit rate with no false alarms). Threshold was defined as the intensity at which the performance measure equaled 0.50, which was usually obtained by interpolation.

### 3. Results

#### 3.1. Behavioral audiogram

The normal mice were first accustomed to maintaining steady contact and drinking from the reward spout for five sessions, during which time the apparatus and procedure were adjusted for the animals (e.g., the optimal spout height was set, the flow rate was adjusted, and the amount of juice that the animals needed per session was determined). The animals were then trained to stop drinking when a 16-kHz tone was presented, a

frequency that previous studies had indicated is in the region of best hearing for house mice (e.g., Berlin, 1963; Heffner 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<sup>J</sup> 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 floor grid. Subsequently, these mice required two to seven conditioning sessions before their lowest threshold was reached. Once trained, the med<sup>J</sup> 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<sup>J</sup> mice did not appear to differ from those of the normal mice as both showed good suprathreshold performance with relatively sharp slopes (Fig. 2).

The audiograms of the mice reveal no difference in absolute sensitivity between the med<sup>J</sup> and normal mice (Fig. 3). Taken together, the animals had an average

threshold of 79 dB at 1.4 kHz, a best threshold of 7 dB at 16 kHz, and a threshold of 75 dB at 90 kHz. At a level of 60 dB, the hearing range of these mice extended from 2.0 kHz to 88 kHz, spanning a range of about 5.5 octaves.

To further investigate their low-frequency hearing, two normal mice were tested at 1 kHz, at which frequency their thresholds were 91 and 93 dB. However, we are reluctant to accept this as a true pure-tone threshold because the 1-kHz signal at these intensities contained noticeable overtones. Specifically, at 3 dB above threshold (95 dB SPL), the signal contained overtones at 2 kHz (25.5 dB) and at 3 kHz (30.5 dB). Although each of these tones was individually below the animal's 2 kHz (69 dB) and 3 kHz (39 dB) thresholds, both frequencies fall within the critical band for mice, which has been estimated at 3162 Hz for this frequency range (Ehret, 1975). As a result, it is possible that these harmonics, particularly the one at 3 kHz, summed with the 1-kHz tone to increase the detectability of the signal. Moreover, whereas the slope of an audiogram generally becomes more steep as the frequency limit of hearing is approached, the slope between 1 and 1.4 kHz appears to be slightly less steep, a potential sign of harmonics. However, we report this point because it puts a lower boundary on the threshold of these mice to 1 kHz, although it is probably an overestimate of their true sensitivity.

### 3.2. Effect of age on sensitivity

The thresholds of two med<sup>J</sup> mice (A and B) and two normal mice (D and E) were retested every few weeks at 3, 16, 32, and 80 kHz to determine if there was any change in sensitivity with age. Fig. 4 illustrates the thresholds, each of which was based on data taken

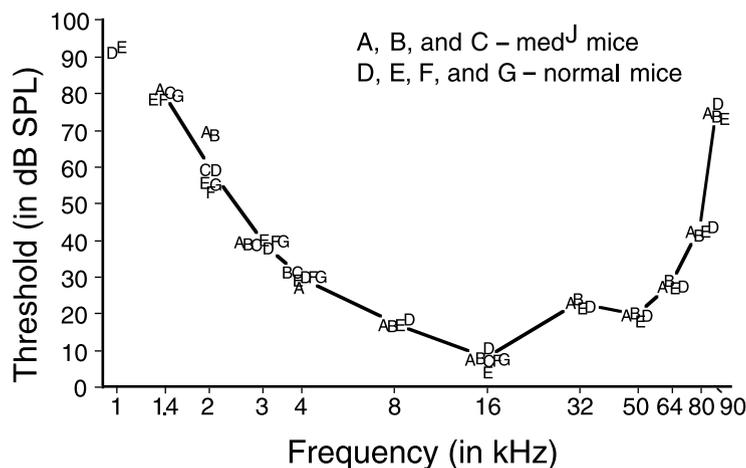


Fig. 3. Auditory thresholds of the seven mice tested in this study. A–C were med<sup>J</sup> mice. D–G were normal controls. The 1-kHz threshold may have been influenced by the presence of overtones (dashed line). (Note that some of the letters indicating the individual mice are displaced horizontally to avoid overlap.)

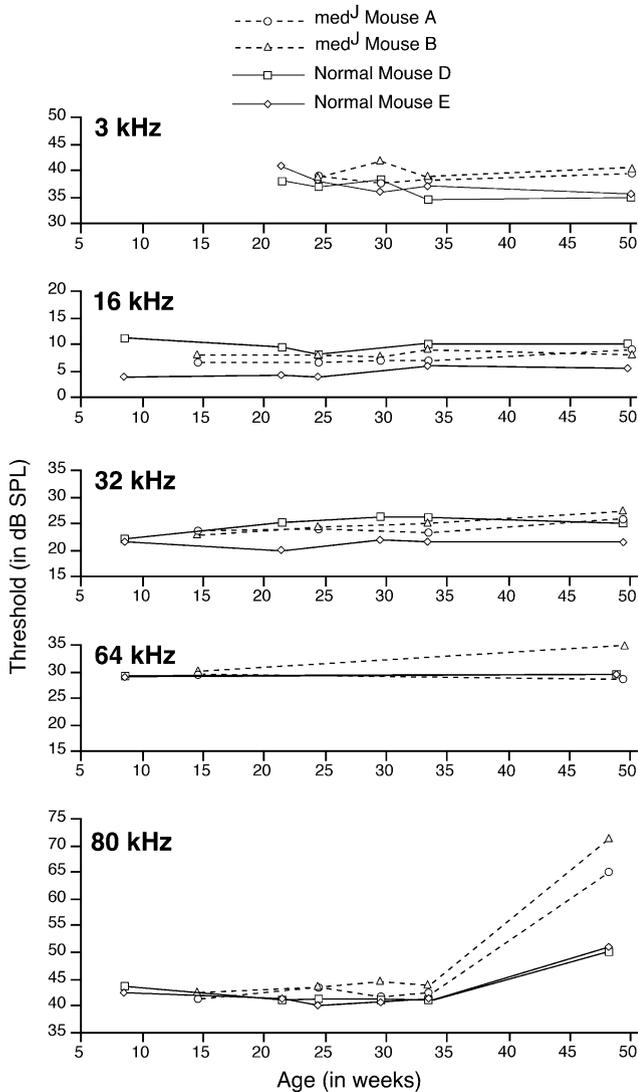


Fig. 4. Thresholds for four frequencies were retested up to 50 weeks of age. Age-related hearing loss was found at 48 weeks for 80 kHz, the highest frequency tested. Thresholds for 64 kHz, determined at 48–49 weeks, did not show a change in threshold.

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 at 16 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<sup>J</sup> 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<sup>J</sup> mice showing a greater loss than their normal littermates.

## 4. Discussion

### 4.1. Behavioral audiometry in mice

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., Heffner and Heffner, 1995; Heffner and Masterton, 1980; Heffner et al., 2001a). Furthermore, requiring an animal to place its mouth on a reward spout fixes its head in the sound field making it possible to accurately specify the sound reaching the ears.

Only minor modifications of the apparatus were necessary to accommodate the med<sup>J</sup> 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<sup>J</sup> mice would not take sufficient 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 difference in testing mice, as opposed to larger animals, is that their low fluid 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.

### 4.2. Effect of med<sup>J</sup> mutation on hearing

Neurons in med<sup>J</sup> mice are deficient in Scn8a voltage-gated sodium channels (Kohrman et al., 1996), channels

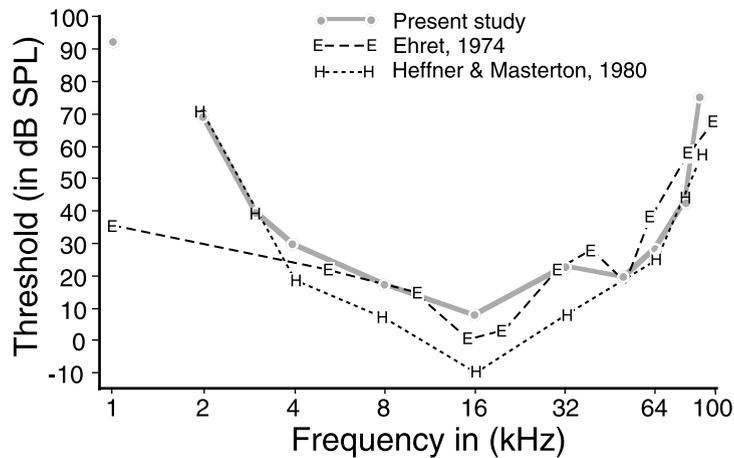


Fig. 5. Behavioral audiograms of house mice. E, audiogram by Ehret (1974) for NMRI mice; H, audiogram by Heffner and Masterton (1980) for wild-caught house mice; gray lines and dots, present audiogram.

which play an important role in the production of action potentials (Llinás, 1988). As a first step in determining the effect of this mutation on hearing, the dorsal cochlear nucleus of *med<sup>J</sup>* 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 firing. This finding suggested that some aspect of their hearing might be abnormal. The results of the present study indicate that the *med<sup>J</sup>* mouse has normal sensitivity, at least for pure tones of 400-ms duration. Whether the animals might have difficulty detecting shorter-duration sounds or whether their ability to discriminate the frequency, intensity, or locus of sounds is impaired remains to be determined.

#### 4.3. The audiogram of the domestic house mouse

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 difference is that the 1-kHz signal used by Ehret contained overtones to which the animals were responding. Indeed, as we found, it can be difficult 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, Heffner 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 differences. That is, strain differences in hearing are the result of mutations that reduce an animal's hearing ability (e.g., Zheng et al., 1999) – there is no known instance of an animal that was successfully bred to have better hearing than its wild ancestors. Thus, it appears that the house mouse, whether wild or domestic, does not hear well at 1 kHz.

Although the threshold of mice at 1 kHz may seem a small point, it is of both theoretical and practical importance. From a theoretical standpoint, the inability of house mice to hear low frequencies places them in the group of mammals that may not use temporal coding for the perception of pitch (Heffner et al., 2001a). From a practical standpoint, researchers need to be aware that mice do not share the low-frequency hearing ability of humans. Thus, in choosing an auditory cue for training mice, a low frequency such as 1 kHz should be avoided because it is virtually inaudible to mice even though it is clearly audible to some other rodents, such as gerbils and hamsters, as well as to humans (Heffner et al., 2001a).

In general, the audiogram of domestic house mice (those without hearing defects) is similar to that of wild-caught house mice in terms of high-frequency sensitivity, low-frequency sensitivity, and frequency of best hearing (Fig. 5). However, wild mice appear to be about 15 dB more sensitive than the domestic mice in

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

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