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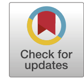
Short communication

Normal audiogram but poor sensitivity to brief sounds in mice with compromised voltage-gated sodium channels (*Scn8a* ^{medJ})



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A B S T R A C T

The Scn8a^{medJ} mutation of the gene for sodium channels at the nodes of Ranvier slows nerve conduction, resulting in motor abnormalities. This mutation is also associated with loss of spontaneous bursting activity in the dorsal cochlear nucleus. However initial tests of auditory sensitivity in mice homozygous for this mutation, using standard 400-ms tones, demonstrated normal hearing sensitivity. Further testing, reported here, revealed a severely compromised sensitivity to short-duration tones of 10 and 2 ms durations. Such a deficit might be expected to interfere with auditory functions that depend on rapid processing of auditory signals.

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1. Introduction

Voltage-gated sodium channels are not only responsible for action potentials in both neurons and muscle, but are also the only sodium channels at nodes of Ranvier (Caldwell et al., 2000). These channels are encoded by at least 10 genes, and several modifications of those genes in rodents have been used to explore the consequences of pinpoint mutations of this complex molecule (e.g., Caldwell et al., 2000; Mackenzie et al., 2009). The *Scn8a*^{medJ} mutation is one such mutation; it reduces Scn8a voltage-gated sodium channels to 10% of their wildtype levels and causes abnormal conduction times, tremors, and motor weakness (Caldwell et al., 2000; Chen et al., 2009; Plummer and Meisler, 1999).

In addition to the obvious motor abnormalities, the *Scn8a*^{medJ} mutation may also affect sensory systems. In the auditory system it causes loss of spontaneous bursting activity in the dorsal cochlear nucleus, leading to interest in its potential effects on hearing abilities (e.g., Chen et al., 1999). However, the audiogram of mice with this mutation (hereafter referred to as medJ mice) is normal throughout their hearing range of 1.4–90 kHz. Moreover, thresholds remain very stable until age-related hearing loss begins to appear at 64 kHz and higher frequencies at 48 weeks of age, only slightly earlier than in phenotypically normal heterozygotes (Koay et al., 2002a). We report here the results of further hearing tests that reveal a marked auditory deficit — specifically, thresholds obtained for 2- and 10-ms tone durations at their frequency of best hearing, 16 kHz, showed an unusually large reduction in sensitivity to brief sounds despite their normal hearing at long durations (400 ms).

2. Methods

The acoustic and behavioral apparatus and procedures are the same as those reported for the audiogram (Koay et al., 2002a; Heffner et al., 2006). Key features are described here.

2.1. Subjects

The *Scn8a*^{medJ} mutation was maintained on a genetic background containing the resistant allele *Scnm* at the master locus, which enabled a small percentage of Scn8a voltage-gated sodium channels to survive and maintain viability of the mice (Kearney et al., 2002; Sprunger et al., 1999). Nevertheless, the conduction of action potentials was severely compromised (Kohrman et al., 1996; Meisler et al., 2001) and these mice exhibit weakness,

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Abbreviations

medJ	Mice with two copies of the *Scn8a*^{medJ} mutation of the *Scn8a* gene for sodium channels (*med*^{J}/*med*^{J})
controls	Phenotypically normal mice that were either heterozygous for the *Scn8a*^{medJ} mutation (*med*^{J}/+), or homozygous wild types (+/+)

tremors, and dystonic postures. Consequently, they dragged their hind limbs as they moved about (Sprunger et al., 1999).

The medJ mice and the phenotypically normal mice tested for comparison, 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 (Buchner et al., 2003). Thus, the medJ mice were *med*^{J}/*med*^{J}, while the phenotypically normal mice (hereafter referred to as control mice) were either heterozygous for the *med*^{J} mutation (*med*^{J}/+), or homozygous wild types (+/+). In other words, all of the mice were from the same homogeneous F1 background and differed only with respect to the *med*^{J} mutation. Three mice (animals A, B, and C) were homozygous *medJ*. Their detection thresholds for brief sounds were compared to those of two control mice (animals D and E) of the same F1 genetic background. All subjects were experienced in auditory tests, and the determination of thresholds for short-duration tones reported here was conducted immediately following their audiograms for longer duration sounds. Thus, these mice were young adults at the time of testing. The procedures were approved by the Animal Care and Use Committee of the University of Toledo.

2.2. Acoustic apparatus

Pure tones at 16 kHz, the frequency of best hearing for mice, were produced using a signal generator (Stanford Research Systems SR770), attenuated (Coulbourn S85-08), and pulsed four times in a 2.0 s trial with a rise/fall time of 1 ms (Coulbourn S84-04, cosine gating). For the 2-ms duration, this meant that full amplitude was not sustained (0 ms plateau). The electrical signal was band pass filtered (Krohn-Hite 3202, 24-dB/octave roll-off, one-third octave above and below the center frequency), amplified (Crown D75), and routed to a piezoelectric tweeter (Motorola KSN1005). The speaker was placed 1 m in front of the mouse at ear level. The electrical signal was continuously monitored on an oscilloscope.

Sound levels were calibrated daily in the position of an animal feeding from the spout using a 1/4-in. (0.64 cm) microphone (Brüel and Kjaer 4939, corrected for free-field with the protection grid on). The output of a preamplifier (Brüel and Kjaer 2669) and measuring amplifier (Brüel and Kjaer 2610) were then routed to a spectrum analyzer (Stanford Research Systems SR770) to check the speaker output for harmonics or distortion.

The maximum stimulus amplitude of a tone pip was determined at the beginning of each test session by observing the output of the Brüel and Kjaer measuring system on an oscilloscope to ensure that the maximum amplitude of the tone at short-durations was the same as it was for long-durations. The measurements showed that the maximum peak-to-peak amplitudes of the tones dropped less than 1 dB at short durations—and when that occurred the voltage was increased to keep the amplitude of the sine wave emitted by the loudspeaker constant.

2.3. Behavioral apparatus and procedure

Testing was conducted in a double-walled sound chamber lined with sound-absorbing foam and carpeting. The mice were tested in a custom cage made of 0.5-in (1.27-cm) wire mesh and mounted on a tripod 1 m above the floor. A 2-mm tube topped with a 5 x 8-mm brass plate served as a reward spout and was placed at a comfortable drinking height for the mice. A mixture of cantaloupe and pear juice served as a reward. The sweet reward helped maintain the health of the medJ mice and caused all the mice to drink more, thereby accumulating more trials during the 20–30-min test sessions. The juice was dispensed at a rate of 7 ml/h from a syringe pump housed in a sound-proof box inside the test chamber.

A contact circuit, connected between the reward spout and cage floor, triggered the syringe pump to dispense a steady flow of fruit juice (which also provided their daily water) when the mice licked the spout. Drinking from the spout served to keep the mice directly facing the speaker in a uniform sound field. An electric shock could be delivered between alternating wires of the cage floor. A 25-watt bulb located below the cage was flashed on and off with the shock. For a drawing of this arrangement, see Koay et al. (2002a).

All of the mice were experienced listeners using the conditioned avoidance procedure. They had been trained previously to respond to 400-ms tone pips in a test to determine their audiogram. Two-second trials were separated by 0.5-s intertrial intervals and approximately 22% of trials contained the train of four tone pulses. When that occurred, the mice had 1.8 s from the beginning of a trial to stop drinking and break contact (a hit) to avoid the shock that accompanied the last 200 ms of the pulse train; the shock light flashed as usual to provide feedback for successful avoidance. Failing to break contact (a miss) resulted in a 200-ms shock from the grid floor. Breaking contact for more than half of the 200-ms period when no tone was present was considered a false alarm. The shock was adjusted for each individual mouse to the lowest level that reliably elicited an avoidance response.

Thresholds were initially estimated by presenting the tones in rapidly decreasing intensities (10-dB steps) until the mouse began to miss. At that point trials were presented in blocks of 6–8 tone trials of a given intensity (and associated trials with no tones) and the intensity of successive blocks of trials was decreased in 5-dB steps. Hit rates were corrected for the false alarm rate and intensity reduced until the hit rate no longer differed reliably from the false alarm rate ($p > .01$). Threshold was defined as the intensity at which the corrected detection measure equaled 0.5, where Corrected Detection = Hit rate − (False alarm rate x Hit rate) (Heffner and Heffner, 1995). Testing for each tone duration continued for at least two sessions or until thresholds stabilized within 3 dB of each other.

3. Results

Fig. 1 shows the threshold elevations for 16-kHz tones of both the control mice and the medJ mice as a function of duration. At 400-ms duration, thresholds for all the mice averaged 7 dB (control mice range 7–7.5 dB; medJ range 6.5–8 dB), closely replicating their previously published thresholds (control mice range 5–10 dB; medJ range 7–8 dB) (Koay et al., 2002a).

At durations of 2 ms and 10 ms, both groups of mice showed elevated thresholds, but the medJ mice showed much greater increases in thresholds than the control mice. A 10-ms signal duration raised the thresholds of medJ mice 25 dB above the threshold for a 400-ms tone, and a 2-ms duration raised thresholds by 53 dB. In contrast, the control mice showed much smaller threshold shifts of 8 and 23 dB, respectively.

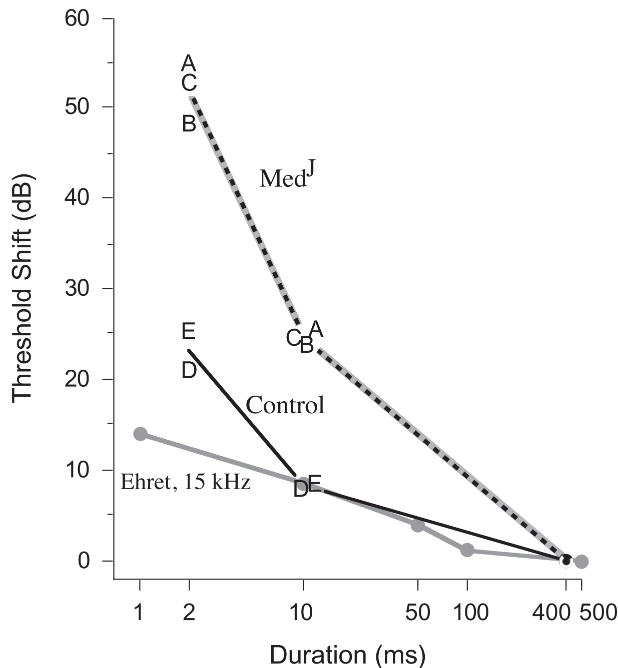

Fig. 1. Threshold shifts at 16 kHz as a function of signal duration for three homozygous medJ mice (A, B, and C) and two control mice (D and E). A further comparison is provided by the results for eight NMRI mice tested at 15 kHz (Ehret, 1976). The medJ mice showed greater threshold shifts than both the control mice and the NMRI mice, but only at short durations of 2 and 10 ms.

4. Discussion

The compromised detection of brief 16 kHz tones by medJ mice can be compared to the results of a study of temporal summation in eight normal mice of the NMRI strain (Ehret, 1976), also shown in Fig. 1. The absolute thresholds for long-duration sounds were consistent among all the mice—4 dB for NMRI at 15 kHz and 7 dB for the mice tested here at 16 kHz. Threshold elevations, above the thresholds for 500-ms signals, for the NMRI mice increased gradually as durations were shortened, showing an elevation of 8.5 dB for 10-ms durations and 14 dB for 1-ms durations.

The control mice reported here showed threshold shifts comparable to those for the NMRI mice for 10-ms tones, and their agreement at both long and intermediate durations is consistent with the earlier finding of normal audiograms for these mice (Koay et al., 2002a). However, the same control mice showed a somewhat greater threshold shift of 23 dB at 2 ms, the shortest duration tested, compared to the 14-dB shift of the NMRI mice at 1 ms.

In contrast, the medJ mice showed much greater threshold shifts for both the intermediate and short-duration sounds at 16 kHz than either the control mice of the same strain or the NMRI mice (Fig. 1). Their ability to detect brief sounds is clearly compromised beyond what might be expected based on strain differences.

Although it is tempting to consider the phenotypically normal mice to be normal in their perception of brief as well as long-duration sounds, Fig. 1 hints at an alternative possibility—that these control mice may also have had a deficit when greater demand was placed on their auditory system. The 8-dB threshold shifts of the control mice for 10-ms tones at 16 kHz were indistinguishable from the 8.5-dB threshold shifts of the NMRI mice at 15 kHz. However, when the duration of the tone was shortened even further to 2 ms, the apparently normal control mice showed a greater threshold shift of 23 dB, somewhat higher than the 14-dB shift of the NMRI mice to the even shorter 1-ms tones (Ehret, 1976). Without the ability to determine whether the two control mice used in this study were homozygous wild types (+/+), or heterozygous for the *Scn8a^{medJ}* mutation (*med^{J}*/+), we cannot know whether this mutation might be responsible, even in the heterozygous state, for a small effect when the auditory system is pushed to extremes of rapid processing. If a much larger group of phenotypically normal but mixed wild types and heterozygotes could be tested, we might see two clusters, one (presumably wild type) with threshold shifts closer to those of the NMRI mice, and another group (presumably heterozygotes) with the larger threshold shift observed here at the 2-ms duration. Alternatively, this could simply be a strain difference between NMRI and the background strains of mice used here or even a difference in the acoustic technology available in 1976 for producing such brief signals without acoustic artifacts.

In summary, these results indicate that a restricted abnormality in nerve conduction time can have a strong effect on rapid auditory processing in the auditory system, as revealed in the ability to detect very brief sounds, while leaving sensitivity to longer sounds unchanged. The hint that there may be a small effect, even in the heterozygous state, could be worth pursuing as it implies that rapid processing of auditory signals could be compromised without other obvious abnormalities.

Finally, these results may have implications for a comparative study of hearing. The only species so far known to exhibit such large threshold shifts with short durations are bats. Specifically, both echolocating and non-echolocating bats have been reported to have threshold shifts in the lower portion of their hearing range as large as those shown by the medJ mice in this report (Koay et al., 2002b). The bats had threshold shifts of 21–72 dB (depending on species) for 10-ms and shorter tones at frequencies of 10 kHz and lower. In contrast, threshold shifts were minimal and typical of other mammals, including other species of bats, at frequencies of 20 kHz and higher. Our results with mice show that a single mutation in a gene affecting an important membrane channel can have a limited, albeit marked, effect on hearing. If such an effect were to prevent certain frequencies from being perceived, it might provide an advantage under circumstances in which those frequencies would interfere with detection of more important sounds—it is conceivable that a similar mutation, if it were expressed only in the auditory system (thereby avoiding severe motor disruptions), could offer an evolutionary advantage and be under positive selection pressure.

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