LATENCY AND AMPLITUDE DIFFERENCES IN ABR WAVE PATTERNS OF KCNA1 NULL-MUTANT AND WILD-TYPE MICE

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Introduction

The auditory brainstem evoked response (ABR) recorded from surface electrodes consists of a regular series of five brief waves and less pronounced additional wavelets that occur within the first 10 ms of stimulus onset. The compact form of any one of these 'peaks' suggests its basis in the near-synchronous firing of a population of homogeneous neurons (Melcher et al. 1996), while the overall development of the series of peaks is thought to reflect the serial and parallel progression of neural transmission from cells of the spiral ganglion and the auditory nerve, through the cochlear nucleus and the superior olivary complex, to the lemniscal nuclei and the inferior colliculus (in mice, Henry, 1979).

The Kv1.1 channel controls a fast acting low threshold rectifying potassium current that limits the duration of subthreshold EPSPs and leads to neural responses that are selective for synchronous synaptic input. In slice preparations pharmacological blockade of this conductance by á-DTX makes octopus cells more excitable and increases their temporal variability (Bal and Oertel, 2001). Also, a loss of synchrony of neural response to stimulus onset was seen in an electrophysiological study of MNTB cells in Kv1.1 KO mice *in vivo* (Kopp-Scheinpflug et al. 2001). The Kv1.1 channel is expressed in spiral ganglion cells (Adamson et al., 2002) and throughout the auditory brainstem (Grigg et al., 2000). The low threshold potassium current is evident in the bushy cells of the ventral cochlear nucleus (Manis and Marx, 1991) and in MNTB cells (Brew and Forsythe, 1995), both of which are thought to make important contributions to the ABR waveform (Melcher et al, 1996).

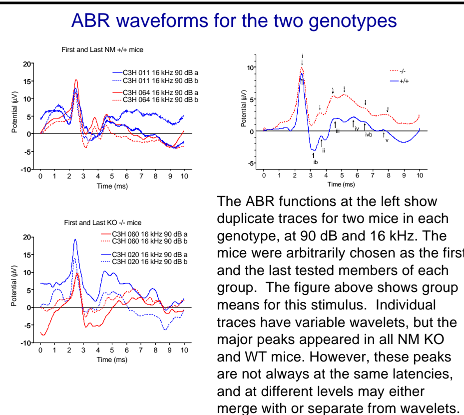
The relative abundance of Kv1.1 in the auditory brainstem, and its apparent critical role in the normal function of cell-types implicated in ABR generation suggest that KCNA1 (Kv1.1) KO mice may show abnormalities in the timing and amplitudes of the ABR component peaks, reflective of deficits in the high-fidelity transmission of auditory information in the mouse brainstem.

Methods

SUBJECTS:
The subjects were 18 wild-type and 12 KCNA1 null-mutant knock-out (KO) mice of the C3HeB strain, born and raised in the Rochester vivarium, the offspring of heterozygotic parents derived from stock originally obtained from the Jackson Laboratories. They were tested at 16-18 days of age.

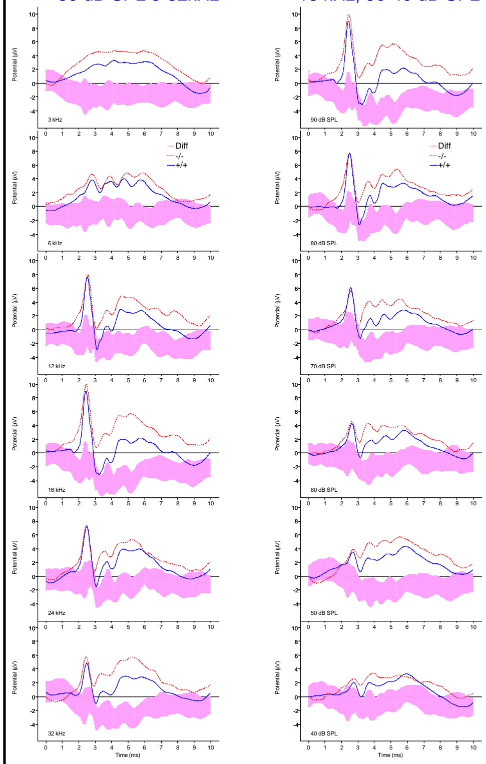
APPARATUS AND PROCEDURE:
ABR audiograms were measured in response to tone bursts (3, 6, 12, 16, 24, 32, and 48 kHz) presented at the rate of 11 bursts per second. Tone duration was 1 ms with a 0.5 ms Blackman-windowed rise/fall time. Stimulus level ranged from 90 dB SPL (80 dB at 48 kHz) to 0 dB, with a replicate at each level. The tone pip stimuli were presented through a leaf tweeter (Panasonic TDH100; effective bandwidth 1-100 kHz) located at 0° azimuth. Stimulus intensity was calibrated using a Bruel & Kjaer ¼" microphone (Bruel & Kjaer Model 4135) placed at the location of the meatal opening of the pinna. The ABR was recorded with subcutaneous platinum needle electrodes placed at the vertex (non-inverting input), right mastoid prominence (inverted input), and right hind limb (indifferent site). EEG activity was differentially amplified (50k or 100k X; TDT HS4 head stage amplifier), and sampled at 50 kHz with an A/D converter (TDT AD1). Each averaged response was based on 300-500 stimulus presentations recorded over 10-ms epochs, filtered at 100 Hz – 3 kHz. The times of the peaks as shown in the figures below reflect a constant delay of 600 us between stimulus presentation and stimulus reception at the ear. Contamination by muscle and cardiac activity was prevented by rejecting data epochs in which the single trace EEG contained peak-to-peak amplitudes greater than 50 µV. During this procedure, a general anesthetic (Avertin® [tribromoethanol]; 5 mg per 10 g body weight i.p.) was used to immobilize the mice, and body temperature was maintained at 38°C with a heating pad. The ABR was recorded in a small sound attenuating IAC chamber.

ABR waveforms for the two genotypes

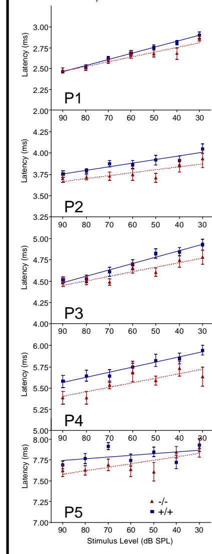


The ABR functions at the left show duplicate traces for two mice in each genotype, at 90 dB and 16 kHz. The mice were arbitrarily chosen as the first and the last tested members of each group. The figure above shows group means for this stimulus. Individual traces have variable wavelets, but the major peaks appeared in all NM KO and WT mice. However, these peaks are not always at the same latencies, and at different levels may either merge with or separate from wavelets.

Group Mean Amplitudes and Differences (+/- 95% CI):



Peak latencies (M +/- SEM)



Summary of Results

The overall wave patterns were the same in both groups, but significant differences are apparent in both the amplitude and the latency data.

Amplitudes and latencies of P1(auditory nerve) were near identical in KO and WT mice, but the KO mice showed a persistent level-dependent positivity. This difference between the groups was largest in the troughs lying between the peaks, and was also present in the early ascent to P1 (see the significant regions in CI functions). Single-trace peak latencies were scored by eye-assisted software (both naïve to group identity) and are subject to confusion between waves and wavelets: none the less, these unbiased data show that later peaks were faster in KO mice (p<.05). Hearing thresholds were similar in the two groups, save that KO mice showed a 10 dB greater sensitivity for P1 at 3 kHz.

Discussion

Modest differences in P1 development suggest a small AN effect, the later differences indicating more substantial VCN bushy cell and possibly SOC involvement. These findings support prior *in vitro* work in showing increased excitability in Kv1.1 KO mice, consistent with a slower return to a baseline resting potential.

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