



Research paper

Kv1.1 channel subunits are not necessary for high temporal acuity in behavioral and electrophysiological gap detection

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ABSTRACT

The Kv1.1 potassium channel subunit, encoded by the *Kcna1* gene, is heavily expressed in the auditory brainstem and is thought to have a critical role in producing the high temporal precision of action potentials characteristic of the auditory system. Our intent was to determine whether temporal acuity was reduced in *Kcna1* null-mutant ($-/-$) mice, compared to wild-type ($+/+$) and heterozygotic mice ($+/-$), as measured by the encoding of gaps in the inferior colliculus by near-field auditory evoked potentials (NFAEP) or behavioral gap detection (BGD) using a prepulse inhibition paradigm. NFAEPs were collected at 40, 60 and 80 dB SPL with gap durations from 0.5 to 64 ms. BGD data were collected using silent gaps in 70 dB noise from 1 to 15 ms in duration. There were no systematic effects of *Kcna1* genotype on NFAEP recovery functions, NFAEP latencies, or the time constant for BGD, but there was a small reduction in asymptotic prepulse inhibition for the longest gap stimuli in $-/-$ mice. Gap thresholds were approximately 1–2 ms across genotypes, stimulus conditions, and paradigms. These data suggest that the neural pathways encoding behaviorally relevant, rapid auditory temporal fluctuations are not limited by the absence of Kv1.1 expression.

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1. Introduction

As reported by Grigg et al. (2000), the voltage-gated Kv1.1 subunit of the Shaker potassium ion channel is heavily expressed in the auditory brainstem, particularly in the octopus and bushy cells of the ventral cochlear nucleus (VCN), the medial nucleus of the trapezoidal body (MNTB), and the medial and lateral superior olivary nuclei (MSO and LSO), with relatively less expression in the dorsal cochlear nucleus and the inferior colliculus (IC). The functional contribution of this subunit in auditory neurons has been studied *in vitro* in the Kv1.1-rich cells of the VCN, particularly the Octopus cells of the posterior VCN. Octopus cells receive inputs from across the tonotopic axis of the auditory nerve projection and Oertel and colleagues have demonstrated that action potentials are most vigorously generated when these inputs are synchronized to millisecond accuracy, and that blocking the Kv1.1 mediated current with α -dendrotoxin degrades this narrow temporal window (Ferragamo

and Oertel, 2002). These properties suggest that Kv1.1 supports the encoding of rapid amplitude modulation, and also that disruption of Kv1.1 channels might interfere with the ability to encode brief, broadband amplitude fluctuations.

In addition, the MNTB slice preparation has been used to elucidate the *in vitro* function of Kv1.1 using selective pharmacological blockade (Dodson et al., 2002) and using the *Kcna1* null-mutant ($-/-$) mouse (Brew et al., 2003; Gittelmann and Tempel, 2006). The anesthetized *Kcna1* $-/-$ mouse has also been used for *in vivo* electrophysiological studies of VCN and MNTB (Kopp-Scheinpflug et al., 2003) and LSO (Karcz et al., 2007). Results from these different preparations agree in showing that neurons lacking functional Kv1.1 channels have weaker onset responses with more variable first spike latency than do $+/+$ neurons and therefore have reduced temporal precision (Kopp-Scheinpflug et al., 2003; Gittelmann and Tempel, 2006). They differ however in one other major respect, that the isolated *in vitro* $-/-$ neuron is hyperactive, with repetitive firing evoked by an electrical pulse that evokes just one action potential in the $+/+$, while the *in vivo* $-/-$ neuron is hypoactive, with a higher threshold and a lower asymptotic firing rate to acoustic input.

Studies of the awake and behaving *Kcna1* null-mutant have focused on motor activity, specifically on the maturation of ataxia and epileptic seizures that are ultimately lethal in these mice (Smart et al., 1998; Rho et al., 1999). Kv1.1 expression is high in

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the CA3 pyramidal neurons of the hippocampus, and seizure activity is presumed to result from the demonstrated hippocampal hyper-reactivity in the *Kcna1* null-mutant mouse (Smart et al., 1998).

The present experiments are part of a research program intended to assess the contribution of potassium ion channels to auditory function, by studying the pattern of sensory-behavioral differences and similarities between particular null-mutant, heterozygote, and wild-type mice for genes expressing different members of these channel families. Ongoing experiments examine these behavioral effects in sound localization tasks (e.g., Allen et al., 2003b; Brew et al., 2005, 2006; Ison et al., 2007b; Karcz et al., 2008), and these show that *Kcna1* null-mutant mice have deficits in these tasks, which are consistent with the *in vitro* and *in vivo* demonstrations of their loss of temporal precision in the neural pathways subserving binaural function. The experiments reported here use gap detection to examine temporal acuity in *Kcna1* null-mutant mice to test the hypothesis that these mice show deficits in millisecond-scale temporal acuity, as measured in behavioral and evoked potential experiments. Gap detection is a well accepted paradigm in humans and in laboratory animals for measuring one aspect of auditory temporal processing, namely, the ability to detect a single brief gap between two flanking markers. Gap detection thresholds lie typically between about 2 and 4 ms in humans and in various species of laboratory animals (see a brief review in Ison et al. (2005)), the values varying in part with the spectral composition of the noise markers, the level of the stimuli, and other methodological details. Here we report that mice lacking the *Kcna1* gene are not measurably different from heterozygous and wild-type mice in their temporal acuity, measured in gap detection paradigms using either IC near-field auditory evoked potentials (NFAEP), following Allen et al. (2003a), or behavioral gap detection (BGD), following the procedures of startle reflex audiometry (Young and Fechter, 1983; Barsz et al., 2002; Ison et al., 2005). We do find reduced prepulse inhibition to the longest duration gaps for the *Kcna1* null mice, however with no IC correlate of this effect, the neural basis of this deficit remains uncertain.

2. Materials and methods

2.1. Subjects

This study was performed using C3HeB.129S7(B6)-*Kcna1*^{tm1Tem} mutant mice, born and raised in the vivarium at the University of Rochester from heterozygote breeding stock (+/- for the *Kcna1* gene) initially obtained from The Jackson Laboratory (Bar Harbor, ME, USA). DNA was extracted from tail snips at about PND 18, and the mice were categorized as +/+, +/-, and -/- using the protocols described at <http://depts.washington.edu/tempelab/Protocols/KCNA1.html>. A total of 71 mice were used in the two experiments reported here: 41 in the behavioral test, 14 +/+, 17 +/-, and 8 -/-, tested on at a mean (SD) age of 32(+/- 2) days; and 31 in the evoked potential test, 10 +/+, 10 +/-, and 11 -/-, tested between 28 and 42 days of age. The ambient noise level in the colony room was 40 dB SPL at 2 kHz and decreased linearly to 25 dB SPL at 24 kHz on a log-frequency scale. Prior to experimentation mice were screened for obstructions of the external auditory meatus and peripheral sensitivity was assessed via an ABR screening test (as described in Ison et al. (2007a)) on about PND 18 and had age- and strain-appropriate hearing thresholds. The overall means ABR thresholds across all test frequencies from 3 kHz to 48 kHz were near identical in the three groups of mice, differing from the overall mean by just +/- 1.5 dB SPL. All procedures were approved by the University of Rochester Committee on Animal Resources and were in accord with the regulations of the Public Health Service and the federal Animal Welfare Act.

2.2. Near-field auditory evoked potentials (NFAEP)

The methodological details of the NFAEP measurement are as described in Allen et al. (2003a), including the surgical procedures, recording and data acquisition and analysis. In this test the animal's detection of a quiet gap between two noise bursts is revealed in the presence an auditory potential evoked by the second noise burst.

For surgery mice were anesthetized with Avertin (200 mg/kg), the cranium was exposed and small, threaded metal tube was affixed to the surface of the skull using cyanoacrylate. A 0.5 mm diameter hole in the skull exposed the IC and a tungsten wire was implanted in the skull adjacent to the head post and in contact with the dura mater to act as the indifferent electrode. The hole was covered with bone wax until the recording session was begun, typically 24 h later.

For recording sessions, mice were lightly tranquilized with Taractan, the head was immobilized by securing the head post to a metal arm affixed to the stereotaxic frame, and a tungsten electrode (either monopolar: 20–50 μ m tip, or bipolar: 50–150 μ m tip separation, (100–250 k Ω impedance) was manually lowered onto the surface of the brain, then to a depth of 500 μ m using a micropositioner (Newport Klinge Micropositioner 705). Neural activity was recorded between tungsten and indifferent electrodes, amplified (10,000 \times), and band-pass filtered (10 Hz–3 kHz) using a TDT Bio-amplifier (HS4/DB4) then digitally sampled (40 kHz) using a TDT AD2. Tucker–Davis Technologies (TDT) hardware and software were used to digitally generate (SigGen32) and present (BioSig32) the stimuli (100 kHz sampling rate, TDT DA3). Stimuli were attenuated (TDT PA4), amplified (Kenwood 620), and broadcast from a Panasonic Model 203 leaf tweeter, which had a transfer function that varied by less than 6 dB from 2 to 50 kHz. The speaker was placed 15 cm away from the left ear at 60 degrees from the midline in the contralateral hemisphere from the recording.

Gap stimuli consisted of paired broadband noise bursts, NB1 and NB2, 50 ms in duration (0 ms rise/fall time), separated by a silent gap. The gap ranged in duration from 0.5 ms to 64 ms. Each noise burst pair was presented 100 times in a series, and the series was replicated. Presentation rate varied such that the interval between NB2 offset and subsequent NB1 onset remained constant at 250 ms. Gap stimuli were presented at 80, 60, and 40 dB SPL for each gap duration. The presentation order of gap durations was randomized between-subjects, however a “no gap” control condition (0 ms gap) initiated each series.

As seen in Fig. 1, the morphology of the IC NFAEP to stimulus onset is stable across the recording session, but varies between animals (see Allen et al., 2003a). We found no consistent effect on waveform morphology of genotype. A semi-automated LabView program was used to compute the RMS amplitudes of the NB1 and NB2 NFAEP, as well as the latency of the first peak of the NB2 response, calculated with respect to NB2 stimulus onset. Amplitude recovery functions were derived for each mouse and for each stimulus level by normalizing the NB2 amplitudes to the NB1 amplitudes, in order to measure the fraction of recovered amplitude at each gap width. These normalized values were plotted as a function of gap duration at the three intensities for each subject (Fig. 2, LH). NB2 Latency recovery functions were created with respect to the same stimulus parameters (Fig. 2, RH). Prior to the gap detection testing, amplitude-level functions were gathered between 10 and 80 dB at tone frequencies of 3, 6, 12, 16, 20, 24, 32, 36, and 48 kHz: no group differences were apparent ($F < 1$).

2.3. The behavioral test for gap detection

The methodological details of the behavioral gap test are as described in Ison et al. (2005). In this test the animal's detection of a

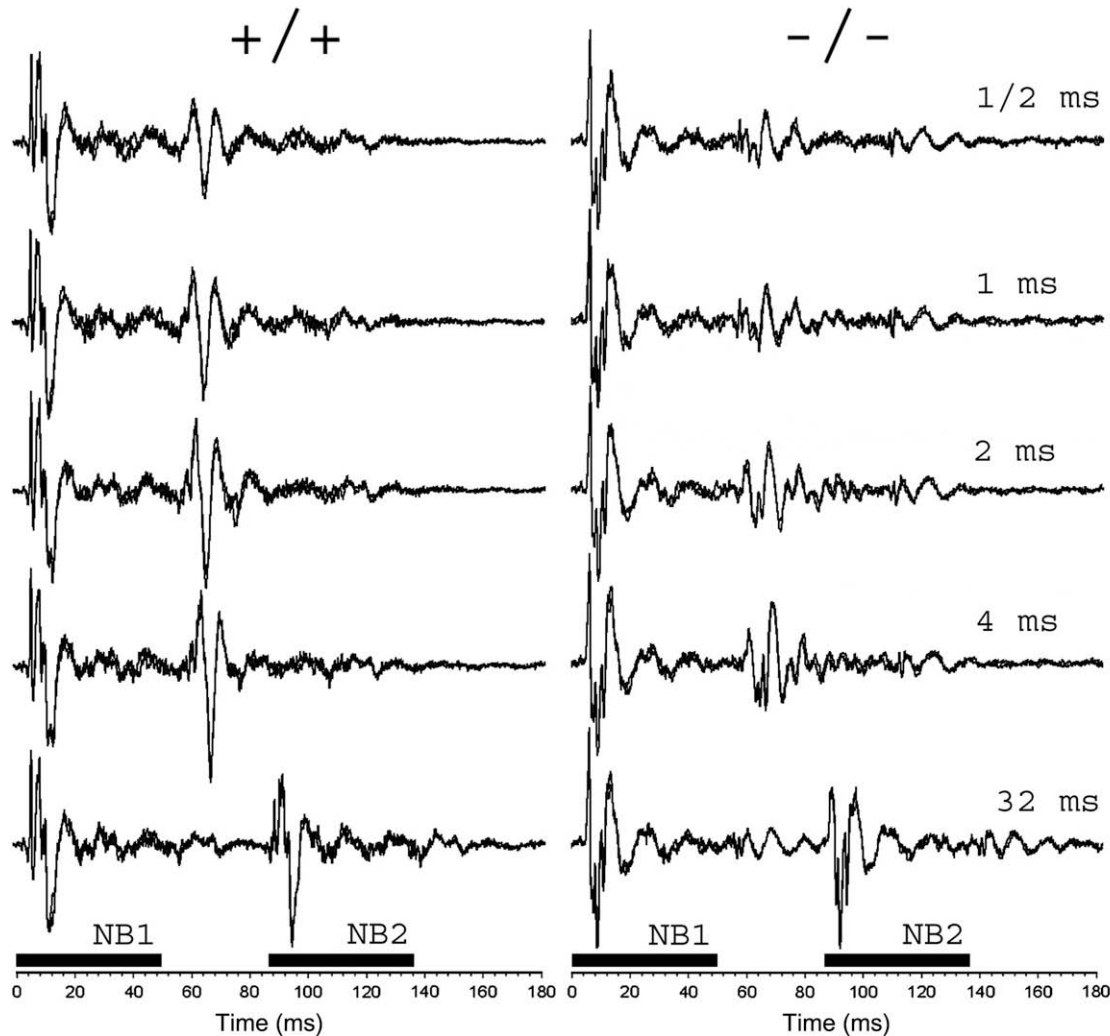


Fig. 1. Examples from individual *Kcna1* *+/+* (left waveforms) and *-/-* (right waveforms) mice of NFAEPs evoked by two broadband noise bursts presented at 80 dB SPL and separated by silent gaps of 0.5 to 32 ms. Each noise burst elicits a complex onset response lasting ~30 ms, and also an offset response at the end of the burst. With increasing gap duration the onset response to NB2 becomes larger and more like that to NB1. Following each onset response there is a period of tonic activity which lasts for the duration of each NB and is characterized by greater activity than what is present during the quiescent period visible following NB2 offset.

quiet gap in an otherwise continuous background noise is revealed as a difference in the amplitude of a reflex evoked by a relatively intense startle eliciting stimulus that is presented 60 ms following the gap, compared to a no gap condition. The mouse is placed for testing in a wire mesh oval-shaped cage mounted on a suspended acrylic platform to which an accelerometer is attached. Testing was conducted in an anechoic chamber. The accelerometer is sensitive to the vertical force exerted by the startle reflex, and to quantify the amplitude of a startle reaction its output is integrated over a 100 ms period beginning with onset of the startle eliciting stimulus. The source for the startle stimulus, which was a 110 dB SPL wide band noise (WBN) 20 ms in duration (that to the human ear is about equivalent to snapping the middle finger into the palm at a distance of 5 cm), and for the background noise (a 70 dB SPL WBN carrier) was a digital signal generator (RP2.1, Tucker-Davis Technologies, Gainesville FL). The intervals between successive startle stimuli are on average 20 s apart, and the trials are presented in 11 blocks of 12 trials, each block containing (a) two presentation of a control stimulus alone (0-ms gap) to provide a baseline startle amplitude; (b) one presentation of each of nine non-zero gap durations (1, 2, 3, 4, 5, 6, 8, 10, and 15 ms) these beginning 60 ms prior to the startle stimulus to provide a measure

of gap detection; and (c) one trial in which background activity was measured for 100 ms with no preceding stimulus, this being used to assess the effectiveness of the startle stimulus. Two mice, one in the *+/+* group and one in the *-/-* group were eliminated from the gap detection analyses because their responsiveness on startle trials was not significantly different from their levels of background activity in the absence of any stimulus.

2.4. Data analysis

The normalized NFAEP responses and latencies were each subjected to a 3-way repeated measures mixed ANOVA, while the relative startle reflex amplitudes were subjected to a 2-way repeated measures mixed ANOVA, (SPSS, Version 15, with genotype as the between-S variable and gap duration as the within-S variable in both experiments, and stimulus level as an additional within-S measure for the NFAEP). The Huynh-Feldt correction for non-homogeneity of inter-condition correlations was used, and partial-eta-square (η_p^2) provided an indication of effect size in terms of “proportion of variance accounted for” by any combination of independent variables. Post-hoc power analysis was conducted using G*Power 3 (Faul et al., 2007).

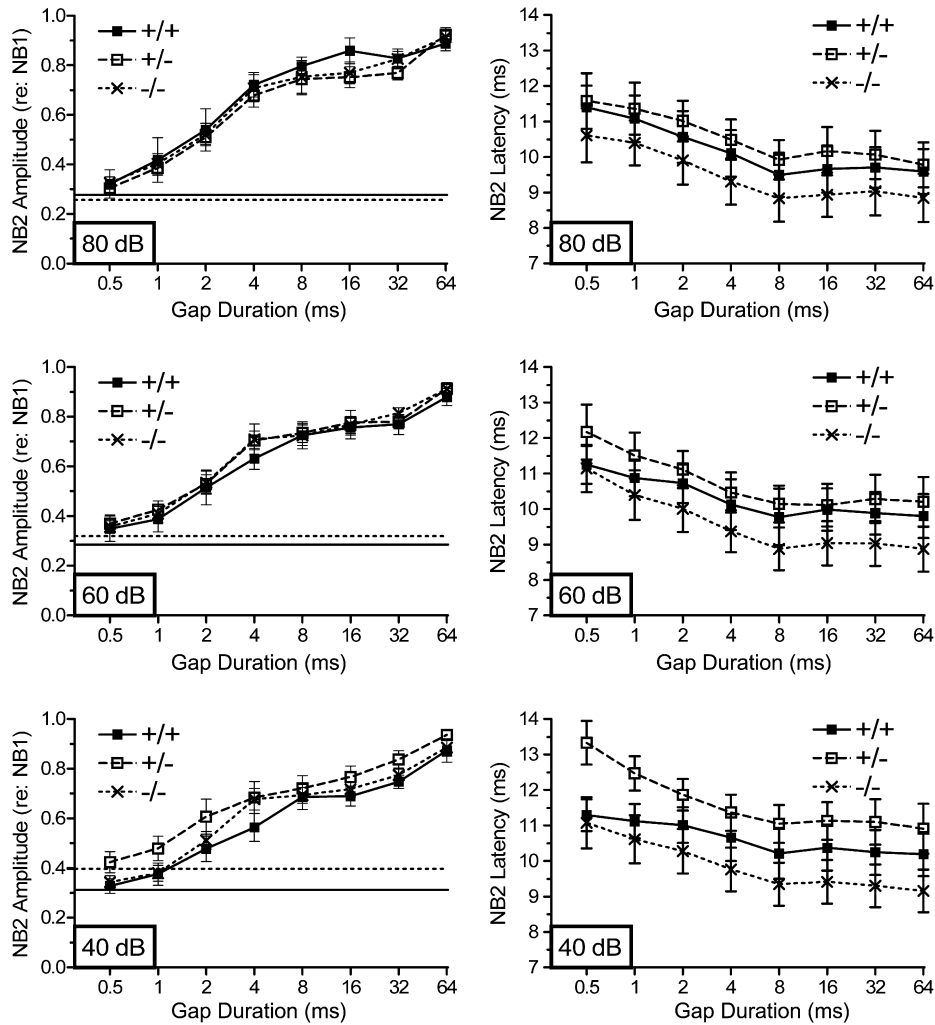


Fig. 2. The left panel shows NFAEP amplitude recovery functions plotted as a function of gap duration. Normalized RMS magnitude was calculated by dividing the amplitude of the response to the second noise burst in the stimulus pair to that of the first. NFAEPS were elicited by the gap carriers present at 80 (top), 60 (center), and 40 dB SPL (bottom), from wild-type (+/+; $n = 10$), heterozygous (+/-, $n = 10$), and null-mutant (-/-, $n = 11$) mice. Horizontal lines represent the “no gap” activity level, again expressed as a proportion of the NB1 amplitude: solid line is combined +/+, -/-, dashed line is +/- . The right panel shows the response latency of the first component of the NB2 response relative to stimulus onset, with the same parameters as for the amplitude recovery functions. All error bars are SEM.

3. Results

3.1. NFAEP

The left hand column of Fig. 2 depicts the mean NFAEP amplitude recovery functions with increasing gap duration for each genotype at each noise level expressed as a proportion of the onset response to NB1. The Wild-type and Null-mutant groups had similar “no gap” RMS activity at each sound level, which reflects ongoing tonic activity to the noise stimulus, and the RMS level of this activity is shown as the solid horizontal line on each plot. This background activity is shown separately for the heterozygous group as dashed horizontal lines, since for this group the control measure was elevated for the 40 dB SPL presentation level, reflecting reduced signal to noise in these recordings. The recovery functions are monotonic increasing with increasing gap duration and rise most rapidly prior to 8 ms; they do not appear to differ across stimulus level, and they are near identical for the three genotypes within each level, save for the heterozygous group at 40 dB, owing most likely to poorer signal to noise for these recordings. The overall ANOVA of these data resulted in a significant main effect only for gap duration, $F(7/196) = 253.19$, $p < 0.001$, $\eta_p^2 = 0.70$. The main effect of

genotype provided $F(2/28) = 0.22$, $\eta_p^2 = 0.02$, and that of level provided $F(2/56) = 0.65$, $\eta_p^2 = 0.02$. However, there was a significant interaction of level and duration in these data, $F(14/392) = 129.09$, $p < 0.001$, $\eta_p^2 = 0.82$, this resulting because the recovery rate for short gaps was faster at higher stimulus intensities. A post-hoc ANOVA of the 0.5 ms gap compared to the “no gap” control condition provided a significant difference, $F(1/28) = 53.85$, $p < 0.001$, $\eta_p^2 = 0.66$, with no apparent effect of genotype ($F < 1$).

The right hand column of Fig. 2 shows the latency of the earliest component of the NB2 evoked response, plotted with increasing gap duration for each genotype at each noise level, calculated with respect to the onset of NB2 presentation. Latencies decrease with increasing gap duration, and reach their asymptotic value by 8 ms at each stimulus level and for each genotype. Latencies become longer at lower stimulus levels, and are fairly similar for the three genotypes within each level, although not to the degree that the amplitude recovery functions are, owing to the larger variability in this measure. This results from heterogeneity in the NFAEP waveform between animals. The overall ANOVA of these data provided a significant main effect for gap duration, $F(7/182) = 90.2$, $p < 0.001$, $\eta_p^2 = 0.776$, and level, $F(2/52) = 31.2$, $p < 0.001$, $\eta_p^2 = 0.545$. The main effect of genotype provided $F(2/$

26) = 1.19, $\eta_p^2 = 0.084$ and was not significant. There was a modest but significant interaction of level and genotype in these data, $F(4/52) = 4.64$, $p = 0.005$, $\eta_p^2 = 0.263$, this resulting because latencies for the heterozygous group showed much slower latencies at 40 dB than the other two groups, as can be seen in Fig. 2. Note, however, that in this condition the raw data showed reduced signal to noise even for longer gap durations, as evidenced by the amplitude recovery functions. The overall pattern of results indicates no effect of *Kcna1* genotype on IC gap encoding as measured with this technique.

3.2. Behavioral measure of gap detection

Fig. 3 shows the increase in startle prepulse inhibition as a function of gap duration for each of the three genotypes. The overall appearance of these data is typical for young mice, in their increasing rapidly with increasing duration up to about 4 to 5 ms, followed by their reaching an asymptotic plateau by 8 ms. The course of the initial increase up to 5 ms gap duration was very similar in all three groups, while the asymptotic levels at 8 ms and beyond appeared to be reduced in the null-mutant mice compared to the +/+ and +/- mice. The inhibitory effect of the 1 ms gap failed to reach significance at the 0.05 level for the three groups combined, $t(37) = 1.99$, $p = 0.054$, while the effect of the 2 ms gap was highly significant, $t(37) = 7.67$, $p < 0.001$: there were no significant differences between the three groups at these two gap durations ($F < 1$). In order to better locate a potential significant effect of *Kcna1* deletion in the overall data, we increased the power of the analysis by combining the +/+ and +/- groups and then compared this larger sample against the -/- group. The main effect of gap duration was significant, $F(8/288) = 9.44$, $p < 0.001$, $\eta_p^2 = 0.21$, but the main effect of genotype was not significant, $F(1/38) = 2.24$, $p > 0.1$, $\eta_p^2 = 0.06$. There were also significant linear and quadratic trends for gap duration ($p < 0.001$) and in addition there was a modest but significant interaction between the linear trend and genotype, $F(1/36) = 4.74$, $p = 0.04$, $\eta_p^2 = 0.12$. Subsequent overall analyses of the 1 to 5 ms gap durations found no effect of genotype or of genotype interacting with duration ($F < 1$), while at the longer 8 to 15 ms durations there was a modest but significant effect of genotype, $F(1/36) = 4.81$, $p = 0.04$, $\eta_p^2 = 0.12$, and this can be seen in Fig. 3 as the reduced amount of asymptotic prepulse inhibition for the -/- mice generated by these longer gaps. The exponential association curves of Fig. 3 have mean asymptotic PPI (SEM) for +/+, +/-, and -/- of 0.42 (0.03), 0.40 (0.03), and 0.28 (0.04), while the association half lives are, respectively, 2.19 (0.44), 1.58 (0.35), and 1.24 (0.63) ms. The mean amplitudes (SD) of the baseline startle reflex for +/+, +/-, and -/- groups in order were 557 (136), 604

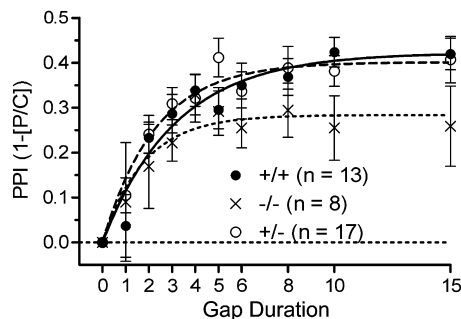


Fig. 3. Behavioral gap detection assessed via the inhibitory effect of brief quiet gaps on the amplitude of the acoustic startle reflex expressed relative to the control baseline amplitude as a function of gap duration, for wild-type (+/+, $n = 13$), heterozygous (+/-, $n = 17$), and null-mutant (-/-, $n = 8$) mice. All error bars are SEM. Curves show one-phase exponential fit, highlighting similar time constants for the three datasets, but lower asymptotic inhibition for the -/- group.

(169), and 546 (215), which were not significantly different, $F < 1$, $\eta_p^2 = 0.03$. While there is reduced PPI in the null-mutant mice for long gap durations, there is no apparent difference between the genotypes for detecting the shorter gap durations that would suggest a deficit in auditory temporal acuity *per se*.

4. Discussion

Prior *in vivo* and *in vitro* electrophysiological research comparing wild-type with null-mutant *Kcna1* mice (Brew et al., 2003; Kopp-Scheinflug et al., 2003; Gittelman and Tempel, 2006) have shown that the Kv1.1 ion channel that is the product of this gene appears to be important in minimizing the temporal spread of evoked onset responses, and thus, may be important for sensory processes that depend on temporal precision. Indeed, ongoing behavioral studies (Allen et al., 2003b; Brew et al., 2006; Ison et al., 2007b; Karcz et al., 2008) indicate that *Kcna1* null-mutant mice exhibit the deficits in sound localization that might be expected given the *in vivo* electrophysiological effects observed in brainstem nuclei that mediate binaural processing (Kopp-Scheinflug et al., 2003; Karcz et al., 2007). The present experiments were designed to determine whether these behavioral and electrophysiological deficits in *Kcna1* null-mutant mice would also impact monaural auditory temporal acuity on the millisecond-scale, as measured in two different measures of gap detection, NFAEP and BGD. We found no evidence to suggest that *Kcna1* deletion impairs threshold gap detection, while some evidence from the behavioral experiment that there may be altered processing of longer gaps.

The absence of near-threshold temporal acuity differences in these measurements is not due to hearing deficits in the C3HeB strain that served as the background for the gene deletion. This is evidenced by 1) NFAEP amplitude and latency recovery functions for all three genotypes that are comparable to those previously reported for 3 month old CBA/CaJ mice (Allen et al., 2003b) and chinchillas (Guo and Burkard, 2002); 2) these mice have behavioral gap detection functions that mirror those of young CBA mice (Barsz et al., 2002), and while there was a small deficit in reflex inhibition at long gap durations, it is at least possible that this effect results from a motor control deficit (e.g., Smart et al., 1998), rather than impaired stimulus salience for long duration gaps, since no asymptotic effects were present in the NFAEP data; and 3) these mice have excellent hearing thresholds as measured in IC tone-evoked potentials and ABR that are at least as good at comparably-aged CBA mice (Allen et al., 2005). We note that although Patel et al. (2002) did not find a difference in gap response patterns between dorsal and ventral recording locations for NFAEPs measured in young C57 mice, the fact that we used only a single recording depth in this study means that we cannot exclude the possibility that there is an effect of *Kcna1* knockout at lower tone frequencies that would be encoded in the more dorsal regions of the IC. However this possibility seems unlikely given that mice have their best gap detection thresholds for high-frequency gap markers (Ison et al., 2005).

The two experimental techniques employed here to measure gap detection support each other and provide converging evidence that auditory temporal acuity is not impaired in the *Kcna1* -/- mouse. The behavioral gap detection results indicate gap thresholds of 1 to 2 ms for all three genotypes. Asymptotic behavioral performance is achieved by gap durations of 8 ms, this being the same gap duration the leads to asymptotic NFAEP latency, and is also the end-point of the rapid portion of the amplitude recovery function. Similar two-noiseburst gap paradigms have been successfully employed in human psychoacoustics (e.g., He et al., 1999; Barsz et al., 2002; Snell et al., 2002) and they yield comparable gap thresholds. Using this stimulus paradigm and the CBA

mouse for IC single unit electrophysiology Walton et al. (1997) showed that the IC neural correlate of behavioral gap detection threshold is the onset response of phasic units to the onset of the noise marker following the gap (yielding 1–2 ms gap thresholds), not the phasic offset units, or decreased firing of sustained units during the gap. The near-field technique for measuring IC gap encoding was found by Allen et al. (2003a) to be well-matched to the single unit gap encoding reported by Walton and colleagues. Eggermont (1999) recorded neural correlates of gap detection in three auditory cortical fields in the cat, and obtained comparable threshold results to these IC studies. Also, Barsz et al. (2002) demonstrated the close correspondence between gap detection thresholds obtained through human psychoacoustics, mouse PPI behavior, and mouse IC physiology and specifically how these changed with age. Additionally there is an extensive literature establishing the substantive role of the IC in mediating PPI. As reviewed by Swerdlow et al. (1992), Koch (1999), and Fendt et al. (2001), the IC is thought to be a major relay for prepulse inhibition by acoustic prestimuli. This was demonstrated by Li et al. (1998), who showed in rats that excitotoxic lesions of the IC greatly attenuate PPI without otherwise affecting the startle response, and by Carlson and Willott (1996), who showed in C57 mice with progressive high-frequency hearing loss that changes in PPI with hearing loss paralleled changes in the IC representation of tone frequency. These different reports provide converging support for the appropriateness of measuring IC activity as the neural correlate of PPI, and specifically that collicular gap encoding would be expected to correspond well with behavioral measures.

On balance, the present data suggest that if channels containing the Kv1.1 subunit do contribute to gap detection performance, their role is not critical. This finding informs our understanding of the neural mechanisms involved in gap detection that, surprisingly, do not include a strong role for Kv1.1 mediated low threshold K⁺ currents, which after all do facilitate temporal integration and a narrow temporal window for responses of some VCN cell-types, and possibly elsewhere in the ascending auditory pathway, such as the onset-type ICC neurons. Increased variability in neural responses from *Kcna1* knockout, as reported by Kopp-Scheinflug et al. (2003) for MNTB neurons could plausibly be manifest in our recordings as increased mean latency of the NFAEP as well as lowering and broadening of the NFAEP onset peaks. Our broadband gap stimuli presumably elicit responses from a large population of IC neurons, with this potentially masking increased variability in individual neuron responses. However an increase in response latency variability would lead to delayed NFAEPs, and possibly longer gap thresholds, even for a large ensemble of responding units. Owing to the 0 ms lower bound on latency, any increase in variability will skew responses towards a long latency tail, thus shifting the mean response latency to a longer time. Increased latency variability would spread the evoked response activity across time and would result in broader and lower amplitude NFAEP waveforms, which we did not observe in this study. We actually observe an apparently paradoxical decrease in NFAEP latency with *Kcna1* knockout, but note that this difference is not statistically significant, and has a very small effect size. In this regard the NFAEP technique has limitations for examining neural correlates of temporal processing. There is substantial between-subject variability in the waveform of the evoked potential, which renders the measurement of latency much less reliable compared to single unit recordings and future single unit recordings might serve to clarify this possibility. Post-hoc power analysis indicates that the present design had only 20% power to detect a difference in NFAEP latency between +/+ and -/- mice of 0.75 ms. We conclude that the present latency measures concur with the amplitude and behavioral gap measures in demonstrating little or no effect of *Kcna1* genotype on the NFAEP and on gap encoding.

We note also that the lack of an effect of *Kcna1* genotype here on almost all measures of gap detection demonstrates that the gene deletion does not produce global problems in sensory processing that affects performance generally. This possibility is a particular weakness of lesion and knockout models in that an observed change in performance in awake and behaving animals may result from deficits in any part of the recursive network of sensorineural, central, and motor activities that normally support that performance. In this context, it is important to identify behaviors and their neural correlates that are unaffected by gene deletion, so that the origin of deficits for other tasks and stimuli can be better isolated and understood. As such, the lack of an effect on gap detection here is important in validating the utility of the *Kcna1* -/- mouse for studying the specific dysfunction of auditory spatial processing that occurs in these mice.

In conclusion, while the converging behavioral and electrophysiological evidence of the measurements reported here indicate normal temporal acuity *per se*, there may be more subtle effects of *Kcna1* deletion on spike timing and reliability in the inferior colliculus that could be the subject of future single unit studies and indeed such subtle deficits presumably underlie the deficits in behavioral sound localization reported for *Kcna1* -/- mice. Also the current results indicate that the behavioral deficits in sound localization reported for *Kcna1* -/- mice do not arise from a general problem in auditory function, but are perhaps the result of a highly selective dysfunction in binaural neurocomputation.

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