Critical role for cochlear hair cell BK channels for coding the temporal structure and dynamic range of auditory information for central auditory processing

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ABSTRACT Large conductance, voltage- and Ca2+-activated K+ (BK) channels in inner hair cells (IHCs) of the cochlea are essential for hearing. However, germline deletion of BKα, the pore-forming subunit KCNMA1 of the BK channel, surprisingly did not affect hearing thresholds in the first postnatal weeks, even though altered IHC membrane time constants, decreased IHC receptor potential alternating current/direct current ratio, and impaired spike timing of auditory fibers were reported in these mice. To investigate the role of IHC BK channels for central auditory processing, we generated a conditional mouse model with hair cell-specific deletion of BKα from postnatal day 10 onward. This had an unexpected effect on temporal coding in the central auditory system: neuronal single and multimict responses in the inferior colliculus showed higher excitability and greater precision of temporal coding that may be linked to the improved discrimination of temporally modulated sounds observed in behavioral training. The higher precision of temporal coding, however, was restricted to slower modulations of sound and reduced stimulus-driven activity. This suggests a diminished dynamic range of stimulus coding that is expected to impair signal detection in noise. Thus, BK channels in IHCs are crucial for central coding of the temporal fine structure of sound and for detection of signals in a noisy environment.—Kurt, S., Sausbier, M., Rüttiger, L., Brandt, N., Möller, C. K., Kindler, J., Sausbier, U., Zimmermann, U., van Straaten, H., Neuhuber, W., Engel, J., Knipper, M., Ruth, P., Schulze, H. Critical role for cochlear hair cell BK channels for coding the temporal structure and dynamic range of auditory information for central auditory processing. FASEB J. 26, 3834–3843 (2012). www.fasebj.org

Key Words: hair cell-specific BK channel-knockout mice · in vivo electrophysiology · inferior colliculus · discrimination learning

Inner hair cells (IHCs) of the cochlea transform sound into graded receptor potentials (RPs), resulting in release of glutamate, which activates type I afferent auditory nerve fibers. IHC RPs are shaped by depolarizing mechanotransducer currents and repolarizing K+ currents, including a fast-activating K+ current (IK,f) carried by large-conductance, voltage- and Ca2+-activated K+ (BK) channels (1–3); a slow-activating delayed

Abbreviations: ABR, auditory brainstem response; AM, amplitude-modulated; BF, best frequency; BK, large conductance, voltage- and Ca2+-activated K+; BKα, BK channel α subunit; BK−/−, knockout for BKα rat e; BMFR, best modulation frequency for response rate; BMFI, best modulation frequency for temporal synchrony; CR, conditioned response; CS, conditioned stimulus; HC-BK−/−, hair cell-specific knockout for BKα; ICC, central nucleus of the inferior colliculus; IHC, inner hair cell; IK,f, fast-activating K+ current; IK,n, negatively activating K+ current; IK,s, slow-activating K+ current; KCNMA1, potassium large conductance calcium-activated channel, subfamily M, α member 1; MTF, modulation transfer function; OHC, outer hair cell; P, postnatal day; RP, receptor potential; tMTF, rate modulation transfer function; SG, spiral ganglion; SPL, sound pressure level; UCS, unconditioned stimulus; VS, vector strength; tMTF, temporal modulation transfer function

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rectifier K⁺ current (Iₖᵥ; refs. 1–4); and a small, negatively activating K⁺ current (Iₖₐ) carried predominantly by KCNQ4 channels (5–7). Whereas Iₖₐ sets the IHC resting membrane potential at approximately −70 mV, both Iₖᵥ and Iₖₐ contribute to repolarization of the RP, yet Iₖᵥ does so with a faster activation time constant (<1 ms) than Iₖₐ (≥8 ms) (4, 5). Thus, BK channels substantially shape the IHC RP and were expected to contribute to phase locking and be required for normal hearing function (1). Surprisingly, mice with global deletion of BKα subunit (BK⁺/⁻) revealed normal auditory brainstem response (ABR) thresholds (3, 8), at least in the first few months of age.

At an age when BK⁺/⁻ mice still had normal ABR thresholds, their IHCs showed stimulus-induced RPs with an increased direct current component and a reduced and slowed alternating current component compared with those of control mice (2). In addition, the membrane time constant of BK⁺/⁻ IHCs was slowed by a factor of 2 to 3. Despite the increased direct current component of the IHC RP in BK⁺/⁻ mice, both peak and steady-state rates of auditory nerve fiber action potential firing were reduced to almost 50% of control, which points to a direct effect of BK channel deletion on spiral ganglion (SG) neurons (2, 9).

The previously suggested essential role of BK for precise spike timing of high-frequency cochlear signaling (2) therefore has to be interpreted as a combined consequence of BK deletion in IHCs and auditory fibers. Knowledge about how temporal information is transmitted by IHCs is crucial for understanding the interaction between IHCs and auditory nerve fibers during temporal encoding of auditory information, e.g., the periodicity in communication calls in mice (10). By using a mouse model with selective deletion of BK channels in hair cells (HC-BK⁺/⁻), leaving their expression in other peripheral and central parts of the body intact, we examined the importance of hair cell BK channels for central auditory processing.

We tested the acuity of central temporal coding of conditional BK mice by recording responses to temporally modulated sounds from neurons in the central nucleus of the inferior colliculus (ICC) in combination with sound discrimination tasks in a behavioral paradigm. The ICC was chosen because of the ability of ICC neurons to precisely synchronize their discharges to the envelope of sound stimuli, a capability that in general weakens the auditory pathway from the periphery to central levels and that is only rudimentarily present at the level of the auditory cortex (10, 11). Our data point to a crucial role of IHC BK channels for coding the temporal structure and dynamic range of auditory information for central auditory processing.

MATERIALS AND METHODS

Generation of HC-BK⁺/⁻ mice

To establish a mouse line with a tissue-specific deletion of the BK channel α-subunit in hair cells, constitutive heterozygous BK⁺/⁻ mice (SV129 background) were interbred with transgenic mice heterozygously expressing Cre recombinase under the control of the prestein promoter (12, 13). Progenies both carrying one BK mutant allele and being transgenic for prestein-Cre were then crossed with mice carrying two floxed alleles (BKαfloxed/floxed, SV129/C57BL/6 hybrid background) of the BK gene potassium large conductance calcium-activated channel, subfamily M, α member 1 (KCNMA1; ref. 14) to obtain prestein-Cre transgenic BK⁺/⁻ (HC-BK-Ctr) mice. The correct genotype was analyzed by PCR amplification, as described previously (12, 13).

Immunohistochemistry of mouse cochlea and brain

Mouse cochleae were fixed in paraformaldehyde, cryosectioned, and stained as described elsewhere (15, 16). For whole-mount immunocytochemistry, the organ of Corti was dissected and stained as described previously (17).

In the cochlea, a rabbit polyclonal antibody against BKα (APC-021; Alomone Laboratories, Jerusalem, Israel), which was detected with a Cy3-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA, USA), was used. Specimens were embedded with Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA), staining cell nuclei in blue, and photographed using an Olympus AX70 microscope equipped with epifluorescence illumination (Olympus, Tokyo, Japan). The analysis was performed at least in triplicate.

For brain immunohistochemical analysis, on-slide 12-µm cryostat slices from nonfixed mouse brain were preincubated with 10% normal donkey serum in buffer (1% BSA/0.5% Triton X-100/0.05 M TBS). After rinsing with TBS, the slices were incubated with anti-BKα (1:1000 in buffer) and tagged with an Alexa 488-conjugated donkey anti-rabbit IgG (1:1000 in buffer). BK expression was analyzed using a confocal laser scanning microscope [Bio-Rad MRC1000 (Bio-Rad, Richmond, CA, USA) attached to a Nikon Diaphot 300 microscope equipped with a krypton-argon laser (Nikon, Tokyo, Japan)]. The specificity of anti-BKα (1:1000 in buffer) has been described elsewhere (18).

Hair cell electrophysiology

Whole-cell voltage-activated K⁺ currents were recorded in IHCs from postnatal day (P) 18–24 HC-BK⁺/⁻ and the respective control mice (HC-BK-Ctr) using acute preparations of the organ of Corti as described previously (19). The bath solution was 85 mM NaCl, 70 mM lactobionate · NaOH, 5.8 mM KCl, 1.3 mM CaCl₂, 0.9 mM MgCl₂, 0.7 mM Na₂HPO₄, 10 mM HEPES, and 5.6 mM glucose, pH 7.4, 320 mosmol/kg; and the pipette solution was 110 mM potassium phosphocreatine, 20 mM KCl, 10 mM Na⁺ phosphocreatine, 5 mM HEPES, 5 mM EGTA, 4 mM MgCl₂, 4 mM Na₂ATP, 0.1 mM CaCl₂, and 0.3 mM GTP, pH 7.35, 305 mosmol/kg. Current recordings were performed from IHCs of the apical cochlear turn at room temperature (22°C). Voltage-activated K⁺ currents were elicited by step depolarizations from a holding potential of −80 mV in nominal steps of 10 mV for 20 or 200 ms, followed by a voltage step to −40 mV. Currents were corrected offline for linear leak currents, and voltages were corrected for the uncompensated series resistance and for the liquid junction potential (−9.6 mV). Activation time constants of K⁺ currents were determined by monoexponential fits to the current traces for 2 ms. For full details of data acquisition and analysis, see ref. 19.
Midbrain electrophysiology

ExtracELLular recordings of the spiking activity of neurons in the central nucleus of the ICC of both hemispheres were performed in both genotypes. Surgery and recordings were performed under anesthesia by intraperitoneal injection of a mixture of 65 mg/kg ketamine hydrochloride (Ketavet 100; Pharmacia GmbH, Erlangen, Germany), 6 mg/kg xylazine hydrochloride (Rompun 290; Bayer, Leverkusen, Germany), and 0.2 mg/kg atropine sulfate (Braun, Melsungen, Germany). Additional doses of anesthetics were constantly supplied (25 mg · kg⁻¹ · h⁻¹ ketamine, 2.5 mg/kg xylazine, and 0.1 mg/kg atropine). Body temperature was maintained at 37°C using a remote-controlled heating blanket. Before surgery, ear canals and the tympanic membranes were visually inspected and found to be free of disease. The ICC on both sides was exposed by craniotomy, leaving the dura intact. A 1.5-cm-long rectangular aluminum bar was fixed to the frontal bones with dental acrylic and served as a head anchor for fixation during recordings. Insect needles were inserted into the skull and served as reference electrodes. The animals were then transferred to an anechoic, sound-attenuated chamber. At the end of a recording session (which typically lasted 20–24 h, during which stable recordings could be obtained), animals were sacrificed by an overdose of the narcotic mixture.

Under microscopic control, 2–4 microelectrodes were inserted into the ICC on both sides using a motorized stepping microdrive. Penetrations were made perpendicular to the ICC surface. Unit activity was recorded using a multichannel recording system with 20,000x amplification, band-pass filtering (250-Hz 2-pole low cutoff and 8-kHz 6-pole high cutoff), and 40-kHz sampling at 12-bit resolution per recording channel (multichannel acquisition processor; Plexon, Dallas, TX, USA). Spiking activity of single and multiple neurons was recorded. When possible, single units were identified with a spike sorting algorithm using template matching. Data were displayed online as dot rasters for which the spiking activity as a function of stimulus frequency could be modified separately for each recording electrode. After each single measurement, data were stored separately for each electrode for offline analysis.

Acoustic stimuli [pure tones and 100% sinusoidally amplitude-modulated (AM) tones] were presented free field via an attenuator (PA4; Tucker-Davis, Alachua, FL, USA), an amplifier (Stax SRM-3; Stax Ltd., Saitama, Japan), and an electrostatic speaker (Stax Lambda Nova). The speaker was mounted ~2 cm in front of the animal's head. Speaker output was measured before an experiment using a 0.5-inch condenser microphone (model 4133; Brüel & Kjær, Nærum, Denmark) placed at the position of the animal's head and facing the speaker. The signal from the microphone was amplified (model 2610; Brüel & Kjær), and monitored on a signal analyzer (model 2033; Brüel & Kjær). For frequencies between 0.3 and 20 kHz, the output of the speaker was found to be flat within ±5 dB and without distortion up to 90 dB sound pressure level (SPL). Stimuli were produced using a computer-controlled multifunction generator (DD1, System 2; Tucker-Davis). All stimuli were presented at a constant intensity of 65 ± 5 dB SPL and had a duration of 200 ms (pure tones) or 500 ms (AM tones), with 5 ms rise and fall time. Neuronal activity was also recorded during a 50-ms prestimulus and a 150 ms poststimulus period. Interstimulus intervals used were 0.5 s for pure tones and 0.75 s for AM tones.

For both single-unit and multiunit activity, a unit’s best frequency (BF) was determined from rate functions obtained from pure tone responses, after subtraction of spontaneous activity (which was averaged over all prestimulus periods for a given stimulus set). BF was defined as the pure tone frequency that evoked the highest discharge rate and the frequency response range as the range of frequencies that evoked an excitatory response. An evoked response was defined as a spike rate 3 sd above spontaneous activity. Sharpness of tuning of the isointensity responses was quantified by dividing the BF by the width of the frequency response range 3 dB below BF (Q3dB value).

To quantify responses to AM tones, rate modulation transfer functions (rMTFs; evoked spike rate as a function of modulation frequency of the AM tone; the carrier frequency was set to the BF of the neuron) were calculated from spike counts. To quantify the degree of envelope synchronization (phase locking) of neuronal discharges to AM tones, temporal modulation transfer functions (tMTFs) were generated by plotting vector strength (VS) as a function of modulation frequency. These were calculated from the responses to AM tones, with modulation frequencies ranging from 0 Hz (unmodulated carrier) to 500 Hz in a time window omitting the ON response. The significance of VS values was tested using Rayleigh statistics (20). VS values that were not significant at the P < 0.05 level were set to 0.

From these MTFs, we determined the peaks: best modulation frequency for response rate (BMFr), the modulation frequency that elicited the highest response rate, and best modulation frequency for temporal synchronization (BMFi), the modulation frequency that elicited the best phase locking = highest VS. Furthermore, tuning sharpness of MTFs was determined as an analog to the Q3dB values of the pure tone response (Q3dBAM and Q3dBAM; see, e.g., ref. 11). From tMTFs, we also determined the best VS as a measure of the maximal temporal precision of the phase locking at BMFi and the upper border of phase locking (highest locking, i.e., the highest modulation frequency at which the unit showed significant phase locking).

Shuttle-box discrimination learning

A foot shock [unconditioned stimulus (UCS); 100–200 μA]-motivated shuttle-box avoidance go/no-go procedure was used for discrimination training of two 100% sinusoidal AM tones of different periodicities (20 vs. 40 Hz) but identical carrier frequency of 12 kHz. The lower periodicity always served as the positively reinforced stimulus [conditioned stimulus (CS+)], whereas the higher periodicity served as the negatively reinforced stimulus (CS−); an additional UCS, duration 0.5 s, was applied on false reaction to the CS−. Stimuli had a 400-ms duration with 5-ms rise and fall time and were presented repeatedly at 2 Hz. The SPL of the sounds presented was calibrated to 70 ± 5 dB at the floor level of the shuttle box (microphone type 4135, microphone power supply type 2633, and amplifier type 2656; Brüel & Kjær).

Twenty daily training sessions with 60 trials each (30 randomized presentations of CS+ and CS−; ref. 21) were performed with each animal. Crossings of the hurdle during a 4-s presentation of the CS+ or CS− were counted in each session. If the animal did not cross the hurdle within 4 s after the onset of the CS−, the UCS was turned on, and the CS− presentation continued until the mouse crossed the hurdle, but maximally for 8 s.

Data from BK mutants and control mice (n=14 each) were compared. Within-group differences between conditioned response (CR) + and CR− were tested using a Mann-Whitney U test and served as a measure of learning speed (first day of significant discrimination). Between-group differences in discrimination performance (CR+−CR−) were analyzed using a Mann-Whitney U test and served to compare how well mice learned to discriminate the stimuli.

All experiments were conducted in accordance with the U.S. National Institutes of Health guidelines for use of animals in research and with the ethical principles defined by the German law for the protection of experimental animals. Care and use of the animals and the experimental protocol
RESULTS

Hair cell-specific deletion of the BK channel in mice

In a previous study, it was shown that crossing the PCre line with Rosa26 mice revealed lacZ staining in IHCs and outer hair cells (OHCs) from P10–P12 onward (13), indicating the ability of PCre to delete the floxed BK gene in hair cells from around the onset of hearing. In these mice, few cochlear sections showed lacZ-positive SG neurons (10% of the SG neurons of the specimens; Supplemental Fig. 1 in ref. 13), which was a smaller Cre recombination frequency in SG neurons as reported by Tian et al. (10–20% of all sectioned SG neurons; ref. 12). In mouse IHCs, BK channel protein expression is up-regulated around P12 (1), as shown for HC-BK-Ctr (Fig. 1A). The onset of Cre activity under control of the prestin gene promoter at around P10 (12, 13) prevented BKα protein expression in IHCs of HC-BK−/− mice (Fig. 1B). At the OHC level, HC-BK−/− mice did not show any obvious difference from HC-BK-Ctr mice at P12, with the BK channel being localized within small spots at the base of OHCs (Fig. 1C, D). At P15, however, BKα expression was deleted not only in IHCs but also in OHCs of BK mutants (Fig. 1E, F). This observation indicates that Cre activity inactivates the floxed BK gene in IHCs earlier than in OHCs. Because cell-specific genetic deletion using the Cre-loxP system seldom affects 100% of a particular cell type, we performed whole-mount immunohistochemical analysis using mature cochleae to obtain an estimate of the fractional deletion. At the age of 4 mo, HC-BK-Ctr animals exhibited BKα protein in every IHC (Fig. 1G, I, arrowhead), whereas in HC-BK−/− animals, only a very small fraction of IHCs from the apical cochlear turn showed BKα expression (Fig. 1H). In medial cochlear turns of HC-BK−/− mice, no BKα expression could be found (Fig. 1J), as was observed in midbasal and basal cochlear turns.

In SG neurons, showing no immunoreactivity in global BK−/−, a small number of BKα-immunopositive spots were detected in both HC-BK-Ctr (Fig. 1K) and HC-BK−/− mice (Fig. 1L), indicating that the deletion of BKα in the cochlea was indeed restricted to IHCs and OHCs. In agreement with this result, HC-BK-Ctr and HC-BK−/− mice showed similar BK expression levels in the ICC (Fig. 2A, B), which receives input from both the ipsilateral and contralateral cochlear nucleus, as well as in the cerebellar cortex for a further control (Fig. 2C, D). These findings indicated that the conditional BKα ablation was restricted to cochlear hair cells. This observation was strengthened by the finding that HC-BK−/− mice showed normal motor functions, in contrast to constitutive BK−/− mice exhibiting cerebellar ataxia (14).

Figure 1. BK channel α subunit expression (red) in cochlear sections of HC-BK-Ctr (A, G, E, K) and HC-BK−/− mice (B, D, F, I) and in whole-mount preparations of HC-BK-Ctr (G, J) and HC-BK−/− mice (H, J) at the indicated age and cochlear turn. Note the onset of BKα deletion in IHCs at P12 before the deletion in OHCs at P15 (A–F) and the sporadic BK expression in IHCs of apical cochlear turns (H, arrowhead), but normal expression of BK in SG neurons of HC-BK−/− mice (L). Sections were counterstained with DAPI (blue), highlighting cell nuclei. Scale bars = 20 μm.

Hair cell electrophysiology

Functional confirmation of cell-specific BK ablation was obtained by recording voltage-activated K+ currents in IHCs (Fig. 3) at P18–P24, an age at which BK currents are fully expressed in control animals (4). Typical K+
current families in an IHC of an HC-BK-Ctr mouse at P18 showed rapidly activating, noninactivating outward currents with a maximum amplitude of $\sim$10 nA at 12 mV (Fig. 3A). In contrast, an IHC of a HC-BK$^{-/-}$ mouse expressed a significantly reduced peak current amplitude, and the outward currents showed inactivating behavior at $-5$ mV and greater (Fig. 3B). When

Figure 2. A, B) Representative sections of the central nucleus of the ICC showing no alterations in the BK channel expression profile between HC-BK-Ctr (A) and littermate HC-BK$^{-/-}$ mice (B). C, D) Similar BK expression was also observed in the molecular (mc), Purkinje cell (pc), and granule cell (gc) layers of the cerebellar cortex from HC-BK-Ctr (C) and HC-BK$^{-/-}$ (D) mice. Scale bars = 100 μm (A, B); 50 μm (C, D).

IHC currents of both genotypes within the first 8 ms after depolarization were compared, it became obvious that an extremely rapidly activating current component present in the HC-BK-Ctr IHC (Fig. 3C) was missing from the HC-BK$^{-/-}$ IHC (Fig. 3D). The current present at 1.2 ms after depolarization (dashed vertical lines in Fig. 3C, D) was averaged for each genotype and is shown as a function of voltage in Fig. 3E. It represents the K$^+$ current through BK channels or $I_{K,F}$ (1, 4, 19) present in 5 of 5 IHCs from 2 HC-BK-Ctr mice at P19, whereas it was absent in 6 of 6 IHCs from 2 HC-BK$^{-/-}$ mice at P18 and P24. Fits of voltage-dependent activation time constants of monoexponential current activation during the first 2 ms of the depolarizing steps (Fig. 3F) confirmed the lack of any BK current in the HC-BK$^{-/-}$ IHCs (19). Taken together, lack of BK protein in immunohistochemical analysis and lack of $I_{K,F}$ in IHCs indicate functional ablation of BK channels in IHCs of HC-BK$^{-/-}$ mice.

Midbrain electrophysiology

We recorded extracellular responses to pure and AM tone stimuli from the left and right ICC in a total of 232 single units and multiunits in 3 HC-BK$^{-/-}$ mice and 177 single units and multiunits in 3 litter-matched HC-BK-Ctr mice. Because no systematic differences in the responses of single units and multiunits were observed with respect to the response characteristics analyzed here, we present the results of single units and multiunits together. Recordings were made in both male and female mice with ages ranging from P35 to P44. Measures of spontaneous and stimulus-driven dis-
charge rate, temporal locking of action potentials to the temporal structure of the sound stimuli, and tuning sharpness were analyzed (Fig. 4 and Table 1).

This analysis revealed a number of significant differences between the response characteristics of ICC neurons in HC-BK\(^{-/-}\) mutants compared with their HC-BK-Ctr littermates, as summarized in Fig. 4 and Table 1. ICC neurons in HC-BK\(^{-/-}\) mice showed significantly higher spontaneous rates than controls (Fig. 4A), whereas their sound-evoked activity was significantly reduced compared with that in controls (Fig. 4B) for both pure tone and AM tone stimulation. No significant difference was found for absolute spike rates (Fig. 4C). There was no difference in peaks of rate-tuning to AM tones (BMFr, Fig. 4D), indicating that units in mutants were still tuned to the same range of modulation frequencies. Timing sharpness was significantly reduced in HC-BK\(^{-/-}\) mutants, which points to reduced selectivity for sound periodicities with reference to discharge rate (Q\(_{5dB}\)AM, Fig. 4G). The ICC units in the mutants were tuned to significantly smaller BMFt, the modulation frequency that elicited the best response (Fig. 4E). Temporal precision of the phase locking of the responses to the AM sound was better in HC-BK\(^{-/-}\) mice than in controls (best VS, Fig. 4F), although the upper border of modulation frequencies that the units could significantly phase lock to was not changed (Fig. 4F). All data were tested for significance using the Mann-Whitney U test. For P values, refer to Table 1.

**Auditory discrimination learning performance**

We investigated whether the deletion of BK channel protein in IHCs had any effects on the behavior in the discrimination task. We therefore tested possible effects of IHC BK deletion on an auditory discrimination task. A total of 14 HC-BK\(^{-/-}\) and 14 HC-BK-Ctr mice aged from P49 to P73 (mean age HC-BK\(^{-/-}\): 58 d; HC-BK-Ctr: 58 d) at the begin of experiments were trained in a shuttle box in a foot shock-motivated go/no-go paradigm to discriminate between two AM tones, both with carrier frequencies of 12 kHz and modulation frequencies of 20 and 40 Hz. Hair cell-specific BK mutants showed a significantly higher learning performance than their littermate controls (Fig. 5): whereas controls showed significant discrimination between AM tones from training day 8 onward, HC-BK\(^{-/-}\) already showed significant discrimination starting on training day 5 (Mann-Whitney U test, \(P \leq 0.05\)). Furthermore, a direct comparison between mutants and controls revealed that hair cell-specific BK mutants showed significantly better discrimination performance (CR\(^{+} - \text{CR}^{-}\)) from training day 15 on (Mann-Whitney U test, \(P \leq 0.05\)). Finally, linear regression lines of learning curves (HC-BK\(^{-/-}\): \(y=0.79x-0.91, R^2=0.95\); Ctr: \(y=0.34x+0.96, R^2=0.83\)) differ significantly both in steepness (\(P \leq 0.0001\)) and \(y\) intercept (\(P \leq 0.01\)). Thus, hair cell-specific BK mutants were both faster in learning to discriminate the two AM sounds as well as better in the level of discrimination performance reached.

**DISCUSSION**

In the present study, we have analyzed central temporal auditory processing and the discrimination of temporally modulated sounds on a behavioral level in a mouse model with targeted deletion of BK channels in
hair cells. The hair cell specificity of the targeted deletion strategy was demonstrated by lack of BK channel α-subunit protein in IHCs and OHCs, with normal expression in other brain regions. A number of significant differences between the hair cell-specific BK mutants and their littermate controls could be identified, such as the absence of fast BK currents in IHCs, changes in central auditory processing as assessed by unit recordings in the ICC, and altered learning performance in a behavioral auditory discrimination task. These differences shed light on the functional role of the BK channel in hair cells for normal hearing function, because they point to a crucial role of BK in IHCs for defining the temporal fine structure and the dynamic range of auditory information.

### Effects of hair cell-specific BK channel ablation on central temporal auditory processing

Electrophysiological response properties of ICC neurons of the control group were, in general, similar to those reported earlier for the mouse (22, 23). Despite initial assumptions (1), the lack of BK currents in constitutive and hair cell-specific mouse models had surprisingly little overall effects on hearing thresholds. Constitutive deletion of BK channels (BK−/−) increased the membrane time constant and reduced the dynamic range of the IHC RPs (2). IHC membrane time constants were reported to increase from 1.3 ms (BK+/+) to 2.3 ms (BK−/−) at −73 mV at room temperature, corresponding to a decrease of cutoff frequencies from 122 to 70 Hz at 58 mV would amount to 0.7 ms/230 Hz for BK+/+ and 1.8 ms/88 Hz for BK−/− (2). It should be noted that in vivo, IHC membrane time constants will be smaller, and respective cutoff frequencies will be higher, because of the higher body temperature and the existence of the endocochlear potential (24).

As a result of the different cutoff frequencies in both genotypes, differences in the temporal representation of the RP envelope should therefore be expected if the modulation frequencies are above these cutoff frequencies, as indeed is observed in ICC electrophysiology (Fig. 4E). However, the reduced dynamic range of the IHC RPs in the conditional HC-BK−/− mice (Fig. 6A) will result in a steeper input-output function saturating at lower stimulus levels (2). This fact may explain the major findings of the present study, namely the altered electrophysiological responses of ICC neurons and the improved amplitude detection in behavioral experiments in HC-BK−/− mice.

The resting membrane potential of BK−/− IHCs was
not different from that of wild-type IHCs at room temperature in an organ of Corti explant (2), which would not explain an increased spontaneous rate of afferent fibers. However, the higher impedance of IHCs in BK−/− mice at rest would lead to higher depolarizations in response to even minute depolarizing currents. Given that IHC transduction channels are activated up to 13–17% at rest in a low endolymphatic Ca²⁺ concentration (24, 25) and that currents are larger at body temperature and when driven by the endocochlear potential of 150 mV into the IHC, it is likely that IHC resting membrane potentials are more depolarized in vivo than in vitro. Consequently, spontaneous release rates will be increased in HC-BK−/− mice, which would lead to a higher overall excitation level within the auditory system that is reflected in higher spontaneous discharge activity of neurons along the auditory pathway, as observed in the ICC measurements (Fig. 6B, orange arrow). Because the RP of IHCs in HC-BK−/− mice saturates for lower input levels compared with that of controls, amplitude modulation becomes more pronounced in the neural representation. The maximal discharge rate of neurons is always limited; therefore, this higher spontaneous discharge rate comes at the expense of the maximal evoked discharge rate that can be stimulus-driven on top of the spontaneous activity. In consideration of this result, the observed significantly reduced stimulus-evoked activity in ICC neurons in mutants compared with that in controls (Fig. 6B, upward-directed solid orange arrow) will cause a reduced dynamic range left for rate coding. Therefore, this smaller range of possible evoked discharge rates leads to seemingly sharper tuning of responses, as observed for the rMTFs (Fig. 6B, horizontal orange arrows). Nevertheless, this effect does not shift the tuning to other frequencies, because peaks of rMTFs remain unchanged (no effect on BMFr; rate function in Fig. 6B).

In addition to these effects on spike rates of ICC neurons, we also observed effects of BKα deletion on the temporal processing properties of ICC neurons. The impaired phase-locking capabilities of the mutant IHCs were reflected in smaller BMFt in the hair cell-specific BK mutants, namely the temporal tuning optimum was shifted to slower modulations. Consequently, because modulation periods are longer for slower modulations, phase-locked responses can more easily be restricted to smaller phase ranges of the modulation (because a certain phase range now corresponds to a longer time window), leading to higher vector strength values of the AM tone responses as observed.

Figure 5. Learning performance of HC-BK−/− (gray diamonds) and littermate HC-BK-Ctr mice (black dots) in a shuttle-box with a go/no-go paradigm. Animals had to discriminate between two AM tones, with carrier frequencies of 12 kHz and amplitude modulation of 20 and 40 Hz. The discrimination performance given by the difference between CR− and CR+ is plotted as a function of the daily training session. Discrimination performance of HC-BK−/− increased faster than in litter-matched HC-BK-Ctr mice and reached a higher level, as revealed by the data from training sessions 15 to 20. *P < 0.05, **P < 0.01; n = 14/group; Mann-Whitney U test.

Figure 6. Model of effects of hair cell-specific BK channel deletion on electrophysiological response properties of IHCs (A) and neurons in the ICC (B). A) High-frequency sound stimulation (black rectangle, bottom) causes an increased RP in a BK-deficient IHC (red line) compared with an IHC of the control group (blue line). This will lead to an increased spike rate on auditory nerve fibers in HC-BK−/− mice. B) This higher level of excitation may lead to higher spontaneous activity (dotted horizontal lines: red, HC-BK−/−; blue, littermate control) at more central levels of sound processing like the ICC. Assumption of a similar maximal response strength for both genotypes (red and blue dotted horizontal line) results in a smaller dynamic range of stimulus-evoked excitability (upward-directed solid orange arrow) and sharper tuning (horizontal orange arrows) if the tuning and strength of the input rate function (black line) remain otherwise unchanged.
In comparison with the hair cell-specific BK mutants, auditory nerve physiology of constitutive BK−/− mice was strongly affected by a decrease in both peak and steady-state discharge rates recorded in response to saturating tone bursts. Oliver et al. (2) suggested that the decrease in spike rates is contributed by a postsynaptic mechanism, given the fact that SG neurons may contain BK channels and loss of BK channels can decrease neuronal spike rates (14). The observed 20% increase in absolute refractory period observed in constitutive BK−/− mutants is consistent with a similar effect of BK channels in auditory nerve fibers (2).

ICC neurons in HC-BK−/− mice, however, showed significantly higher spontaneous rates than controls, whereas their sound-evoked activity was significantly reduced compared to controls. The higher spontaneous rates in ICC neurons of HC-BK−/− is consistent with the inferred IHC depolarization but has not been observed in constitutive BK−/− mutants in which loss of BK channels in spiral ganglia may decrease neuronal spike rates (2). Thus, even if a deletion of BK channels in 10–20% of spiral ganglia takes place, the phenotype of higher spontaneous rates in HC-BK−/− would be attenuated to some extent, but this would not change one of our main conclusions: in HC-BK−/− mutants the spontaneous discharge rates of the auditory nerve is increased.

It might be argued that IHCs of HC-BK−/− mice are developmentally impaire[d with regard to their ion channel composition, cell size, exocytosis, or tonotopic specialization. A deterioration of IHC specializations along the tonotopic axis in HC-BK−/− mice is unlikely because Ik, A currents carried by BK channels are not tonotopically different in IHCs (26). Moreover, the findings of normal IHC Ca2+ and non-BK K+ currents and cell capacitances in constitutive BK−/− mice (2) suggest that elements maturing before the normal onset of BK expression, such as Ca1,3 Ca2+ channels, are not affected by lack of BK channels in BK−/− mice.

Because the deletion of BK affected IHCs as well as OHCs, a role of OHCs in the observed differences in temporal coding in the central auditory system needs to be considered. First, a possible function of BK channels in wild-type OHCs is repolarization of the membrane potential in acoustic overstimulation and hence protection of OHCs (8, 17). That the lack of this protective function in HC-BK−/− is responsible for the results of the present study is not likely because the stimulation levels used were 70 ± 5 dB SPL at most. Second, K+ current recordings in OHCs suggested a possible role of BK channels in mediating efferent inhibition in the high-frequency cochlear turn (27). Our shuttle-box experiments for assessing the ability of the mice to discriminate AM tones exclusively used carrier frequencies of 12 kHz, which is in the low- to mid-frequency range of hearing in mice and also is the range of their highest sensitivity (28). Third, OHC inhibition elicited by activation of the medial olivocochlear bundle could lead to better discrimination of signals in noise (29–31). The design of our behavioral experiments, however, did not include a signal discrimination task during noise exposure. Thus, BK channels in OHCs should mainly play a role in the high-frequency range not being addressed in the behavioral experiment of this study.

Effects of hair cell-specific BK channel ablation on behavioral performance in a temporal auditory discrimination task

That mice can successfully be trained in an auditory discrimination task using a shuttle-box go/no-go paradigm was first demonstrated by Kurt and Ehret (32). Using a similar approach, in this study we observed improvement in behavioral learning performance in the hair cell-specific BK mutants compared with that in their controls, which is probably linked to the decreased dynamic range of IHC RPs: small-amplitude modulations of the stimulus will lead to relatively large increases in the RP and hence exocytosis; however, at a certain SPL, the RP will become saturated. This higher excitation may then have an enhancing effect on the perceptual salience (possibly in terms of differences in perceived loudness or timbre) of the sounds, leading to a better discrimination within the shuttle-box paradigm, although the stimuli may sound much different to the mutants than to their littermate controls.

Based on these results, we conclude that the lack of BK channels in cochlear hair cells has an effect on dynamic range and signal/noise ratio of responses within the auditory system: because of the higher spontaneous activity within the system, auditory neurons have a smaller dynamic range of discharge rate variations remaining to code for stimulus-evoked excitation. This higher level of excitation may be advantageous for the perception of sounds such as the AM tones used in the learning paradigm, but it should be disadvantageous for the detection of signals in noisy environments.

A human syndrome of coexistent generalized epilepsy and paroxysmal dyskinesia, which is caused by missense mutations of the α subunit of the BK channel leading to a gain of function of the BK channel in heterologous expression systems, has been described (33). Nothing has been reported about the hearing abilities of those patients. However, one would not expect a hearing phenotype similar to that of the HC-BK−/− mice in this study, because the mice had completely lost the BK channels in IHC and OHCs. However, from the mouse model analyzed here, we expect that humans with a partial or complete loss of BK channel function suffer from ataxia (14), increased susceptibility of OHCs to noise trauma (17), and auditory processing disorder.

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