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# L-type calcium channels in the auditory system

## Introduction

Hearing relies on correct mechano-electrical transduction in the inner ear as well as precise neuronal signal transmission and processing along the auditory pathway. The mammalian auditory organ in the inner ear, the organ of Corti (**Fig. 1a**), contains two types of hair cells, inner hair cells (IHC) and outer hair cells (OHC). Whereas OHC locally amplify the effects of low acoustic stimuli thanks to their unique electromotility, IHC are the sensory cells proper. They translate sound-induced changes in their membrane potentials into glutamate release (**Fig. 1b**) causing afferent auditory nerve fibers to be excited (**Fig. 1a, b**), and transmitting neuronal information from the inner ear to the central auditory pathway. Unlike other anatomically less complex sensory systems, the auditory system is characterized by a large number of processing and relay centers in the brain stem. This may most likely be due to the fact that the auditory system does not spatially reproduce the outside world in the organ of Corti, but rather calculates acoustic space via the central nervous system, by computing interaural intensity and time differences in the brain stem. The major part of the auditory brain stem is therefore made up of sound localization circuits.

Due to the tremendous importance of hearing for humans, recent research has increasingly focused on the molecular determinants of auditory development and function. Key contributions to this field of research have been derived from studies with genetically modified mice. According to these studies, two L-type $Ca^{2+}$ channel isoforms, $Ca_v1.3$ and $Ca_v1.2$, play roles in the peripheral as well as the central auditory system, roles that are as important as they are unexpectedly diverse. Below, we will outline the roles of these two isoforms in hearing. $Ca_v1.1$ and $Ca_v1.4$, the two other L-type isoforms, are expressed in the skeletal muscles and the retina, respectively, and have not been found to be functional in the auditory system. They will therefore be disregarded in this paper.

## The multifunctional $Ca_v1.3$ L-type $Ca^{2+}$ channel in the inner ear

The transformation of receptor potentials into graded transmitter release at the synaptic pole of an IHC requires voltage-activated $Ca^{2+}$ channels that open (and close) rapidly and even in response to minor voltage changes, without noticeable tiring. Whereas the presynaptic $Ca_v2.1$ and $Ca_v2.2$ $Ca^{2+}$ channels are found in neurons, in IHC, the $Ca_v1.3$ L-type $Ca^{2+}$ channel triggers $Ca^{2+}$ influx [5, 24] (see **Infobox 1**). The $Ca_v1.3$ channel activates at voltages as low as −65 mV, exhibiting only little voltage and $Ca^{2+}$-dependent inactivation. Unlike the $Ca_v2.1$ and $Ca_v2.2$ channels that activate only when more pronounced potential changes occur, the $Ca_v1.3$ channel is better suited to transform even minor voltage changes into transmitter release. Low $Ca_v1.3$ channel inactivation enables stimulus-triggered, continuous influx of $Ca^{2+}$ ions without fatigue; their rapid activation kinetics guarantees rapid signal transmission.

## $Ca_v1.3$ is essential for auditory pathway cytoarchitecture

The fact that $Ca_v1.3$ has an essential presynaptic function in neurotransmission of the IHC in the inner ear came as a surprise since $Ca_v1.3$—like $Ca_v1.2$—was known to mediate $Ca^{2+}$ influx into the soma and dendrites of neurons. Therefore, the question of whether $Ca_v1.3$ is also expressed, and plays an important role, in the auditory pathway seemed fascinating. Previous experiments in organotypic slice cultures of auditory brain stem had provided evidence that L-type $Ca^{2+}$ channels have an essential function in survival of auditory brain stem neurons. Auditory brain stem slice cultures only survived when $K^+$ concentration in the extracellular medium was elevated (25 mM KCl), which induced slight depolarization [23]. The positive effect of KCl was revoked by addition of L-type $Ca^{2+}$ channel blockers, demonstrating the vital role of L-type channels. The individual roles of $Ca_v1.3$ and $Ca_v1.2$ in this context as well as their functions in vivo remained unclear in this initial study. One of the major difficulties was the lack of selective drugs distinguishing between $Ca_v1.3$ and $Ca_v1.2$ isoforms. Any L-type-$Ca^{2+}$ channels are targeted by dihydropyridines, a class of molecules that includes the frequently used nifedipine and isradipine. Specific antagonists or agonists of each isoform have as yet to be identi-

## Infobox 1: Systematics of voltage-activated calcium channels

Voltage-activated $Ca^{2+}$ channels consist of a pore-forming $\alpha_1$ subunit and accessory β, α2δ, and γ subunits. A total of ten different genes encode the $\alpha_1$ subunit, and, depending on the isoform expressed, $Ca_v1$, $Ca_v2$, or $Ca_v3$ channels will form. The members of the $Ca_v1$ family ($Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$, $Ca_v1.4$) represent the L-type $Ca^{2+}$ channels, primarily expressed in muscle cells, endocrine cells, and the brain. The $Ca_v1.2$ and $Ca_v1.3$ isoforms, chiefly located in the soma and dendrites, are found in the brain. By contrast, presynaptic $Ca^{2+}$ channels, mediating $Ca^{2+}$ influx for transmitter release in neurons, are generally part of the $Ca_v2$ family. In this context, one should mention the $Ca_v2.1$ and $Ca_v2.2$ isoforms conducting P/Q-type and N-type currents, respectively. They are activated by action potentials. The channels from the $Ca_v1$ and $Ca_v2$ families are commonly referred to as high-voltage-activated $Ca^{2+}$ channels (or HVA $Ca^{2+}$ channels) since they are, as a rule, only activated by depolarization with a threshold of at least −45 mV. One should note, however, that $Ca_v1.3$ and $Ca_v1.4$ channels open starting from −65 mV. The $Ca_v3$ family includes $Ca^{2+}$ channels that open at even lower thresholds (low-voltage-activated, or LVA, $Ca^{2+}$ channels). These LVA $Ca^{2+}$ channels primarily have pacemaker function and are not found at synapses.

Mice with ablation of *Cacna1d*, the gene that encodes $Ca_v1.3$, are deaf [24], and humans with a mutation in $Ca_v1.3$, which inhibits channel opening, suffer from congenital deafness [1]. Both the *Cacna1d*$^{-/-}$ mice and humans with *Cacna1d* mutations exhibit accompanying bradycardia, which indicates $Ca_v1.3$ participation in sinoatrial nodal rhythm generation. This genetic disease is therefore known as the SANDD syndrome (sinoatrial node dysfunction and deafness syndrome) [1].

$Ca_v1.3$ channels of mature IHCs cluster around the specialized ribbon synapses (**Fig. 1b**). However, $Ca_v1.3$ channels not only mediate $Ca^{2+}$ influx at IHC synapses, they also have further essential functions during inner ear development, as was revealed by studies with mice. As an altricial species, mice are born blind and deaf and only start hearing around the 12th postnatal day (P12). In the period between birth (P0) and P12, $Ca_v1.3$ currents in IHC are gradually upregulated to reach a transient maximum that equals approx. 300% of the current in IHC of hearing mice (**Fig. 1c**), [3, 16]. Between P0 and the onset of hearing, the $Ca_v1.3$ currents have three functions: (1) They are essential for the generation of $Ca^{2+}$-based action potentials, i.e., for spontaneous activity of IHCs (**Fig. 1c**); (2) in their role as presynaptic channels, they guarantee that spontaneous IHC activity is conducted to the central auditory pathway; (3) they control expression of genes of the mature IHC [5, 15], e.g., expression of the $Ca^{2+}$ and voltage-activated $K^+$ channel (BK channel) gene. Massive expression of BK channels around day P12 turns off the spontaneous activity phase, permitting the generation of sound-induced graded receptor potentials (**Fig. 1c**, [21]). Previously, i.e., during the spontaneous activity phase, IHC are subject to efferent innervation by cholinergic neurons of the olivary complex (MOC neurons) [28]. Via the unusual combination of postsynaptic α9/α10 acetylcholine receptors and the SK2 $K^+$ channel, acetylcholine, the neurotransmitter of cholinergic neurons, triggers inhibitory postsynaptic potentials, thus interrupting IHC activity [10]. This interplay between spontaneous IHC activity and medial olivocochlear efferent inhibition is essential for auditory pathway maturation before the onset of sensory experience [6]. In $Ca_v1.3$-deficient mice, expression of α9/α10 acetylcholine receptors and SK2 channels, whose occurrence in immature IHC is only of short duration, is not turned off. One may therefore assume that absence of $Ca_v1.3$-mediated currents in IHC not only inhibits transmitter release and IHC development, but also affects auditory pathway maturation.

fied, making it difficult to attribute specific functions to individual $Ca^{2+}$ channel isoforms.

In cases such as these, genetically modified mouse strains are an important tool. The CAVNET (MRTN-CT-2006-035367) joint European research project gave us access to several mouse strains with different mutations in the two $Ca^{2+}$ channel isoform genes. Apart from the above-mentioned constitutive *Cacna1d*$^{-/-}$ mice, there is another mouse strain in which point mutation at the dihydropyridine binding site prevents $Ca_v1.2$ modulation by dihydropyridine [29]. This type of mouse is referred to as $Ca_v1.2$ DHP-insensitive. By determining $Ca^{2+}$ currents in the two mouse strains as well as wild-type mice, $Ca_v1.2$ and $Ca_v1.3$ contribution may be identified based on mathematical subtraction. The studies were performed using acute brain slices and taking lateral superior olive (LSO) neurons as an example, as they play an important part in sound localization (**Fig. 2a**). Results show that $Ca_v1.3$ channels contribute 30% of total $Ca^{2+}$ influx into neurons both 1 week before hearing onset and around the time of hearing onset [17]. The idea therefore suggested itself to analyze the developmental significance of $Ca^{2+}$ influx. Immunohistochemical analysis of the synaptic vesicle marker protein VGlut1 in young adult *Cacna1d*$^{-/-}$ mice pointed to aberrant structures of auditory nuclei. The LSO, for instance, lost its kidney bean-shaped form (**Fig. 2b**). Volume measurements of single auditory nuclei in young adult mice revealed drastic reductions ranging between 25 and 59% when the $Ca_v1.3$ isoform was absent (**Fig. 2c**) [12]. This was mainly due to a reduced neuron count, which, in some nuclei, was lower by up to 35%. It was interesting to note that the reduction in volume was largely limited to the auditory system; other cerebral areas with a lower share or no share of auditory neurons (neocortex, tectum, cerebellum) were not modified. Hence, $Ca_v1.3$ seems to be important for the development of the auditory pathway in particular [12].

Unlike the drastic anatomic effects just mentioned, other parameters such as neuron morphology, resting membrane potential, as well as action potential amplitude and duration remained virtually unchanged in the surviving neurons. A significant difference was, however, found in the firing behavior of LSO neurons. While the majority in wild-type mice fired one single action potential after current injection, the majority of LSO neurons in *Cacna1d*$^{-/-}$ mice fired several times (**Fig. 2d**) [12]. As neuronal firing behavior is very much dependent on voltage-dependent $K^+$ currents, we studied LSO neurons for altered function of $K^+$ channels. Electrophysiological and pharmacological experiments revealed changes in $K_v1$ class low-voltage-activated $K^+$ channels. Immunohistological studies eventually provided evidence of reduced $K_v1.2$ expression in auditory brain stem neurons in the absence of $Ca_v1.3$ [12].

## Refinement of auditory circuits requires $Ca_v1.3$

Structured representation of object properties is a basic principle of sensory systems. In the process, stimulus parameters are assessed by the sensory organ for structured reproduction in the subsequent centers. The basic organizing principle of the auditory system is based on the spectral frequency composition of the acoustic signal. Neighboring sensory hair cells in the cochlea are excited by similar frequencies.

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## Abstract

The voltage-activated L-type calcium channels $Ca_v1.2$ and $Ca_v1.3$ mediate $Ca^{2+}$ influx into neurons at the soma or at dendrites, whereas they are not observed at the presynapse. Surprisingly, in the inner ear, $Ca_v1.3$ is indispensable for signal transmission from the presynaptic cochlear inner hair cells to the postsynaptic auditory nerve fibers. Due to $Ca_v1.3$ channel clustering at ribbons, i.e., specific presynaptic structures of the hair cells, they promote $Ca^{2+}$ influx, which triggers calcium-dependent fusion of synaptic vesicles with the plasma membrane. Mutations in *Cacna1d*, a gene that encodes $Ca_v1.3$, result in deafness because release of the neurotransmitter glutamate at the synapses is abolished. Moreover, studies of the auditory pathway have revealed that $Ca_v1.3$ plays an important part in the central auditory system as well. Absence of the channel results in severe changes in auditory pathway cytoarchitecture and in abnormal electrophysiological performance of auditory neurons. Furthermore, developmental refinement of tonotopic inhibitory projections in sound localization circuits is disrupted. These aberrations are associated with abnormal sound processing in the auditory pathway. This goes to show that the $Ca_v1.3$ channel is essential for inner ear functioning as well as auditory pathway development and performance. *Cacna1d* therefore represents a prototypal deafness-associated gene, in which mutations result in both peripheral and central auditory deficiencies. This, in turn, has implications for auditory rehabilitation using cochlear implants that address only peripheral dysfunctions. Exploratory research into the closely related $Ca_v1.2$ isoform points to an important role of this channel in acoustic trauma. $Ca_v1.2$ is mainly expressed in the auditory nerve, but apparently not essential for normal auditory function. Loss of function of the channel, however, does influence the effects of traumatic noise exposure. Loss of this channel induced by noise trauma results in reduced auditory threshold increase—as compared with the control group. This phenomenon points to the fact that $Ca_v1.2$-mediated $Ca^{2+}$ influx is involved in noise trauma-induced damage. Deeper insight into this function might result in new therapeutic approaches.



## Keywords

L-type calcium channel · Ribbon synapse · Inner ear · Auditory brain stem · Cell death


IHC located at the cochlear base are sensitive to high-frequency signals, whereas those located at the apex are sensitive to low-frequency signals. This cochleotopic or tonotopic organizing principle is also encountered in the auditory pathway with neighboring neurons processing neighboring frequencies. During early cerebral development, tonotopic connections are cursorily set up, to be refined in a second developmental step that depends on neuronal activity.

Several refinement processes in other sensory systems are well researched; however, this only applies to excitatory neuronal circuits, whereas studies on inhibitory connections are rare [18]. This is due to the fact that most inhibitory circuits originate from widely dispersed interneurons, which are difficult to study. The auditory brain stem is an important exception insofar as it exhibits an instance of inhibitory projection between two nuclei, the medial nucleus of the trapezoid body (MNTB) and the above-mentioned LSO, that are readily accessible in experiments. At an early developmental stage, single LSO neurons receive weak synaptic connections from more than ten MNTB neurons. During refinement, numerous synaptic connections are cut, with a single LSO neuron losing 75% of its initial inputs. This process is accompanied by a 12-fold increase in the synaptic conductance generated by the remaining inputs (*pruning* and *strengthening*) [20]. Surprisingly, this refinement occurs only a few days after birth, a time when the neonates are still deaf.

One might assume that refinement in the auditory pathway proceeds without acoustic input, in an activity-independent manner, and is only genetically determined. This is, however, not the case as, in infant mice that are still deaf, the cochlea is spontaneously active (cf. "The multifunctional $Ca_v1.3$ L-type $Ca^{2+}$ channel in the inner ear"), with this activity resulting in neurotransmitter release in the auditory brain stem. It has been shown that the synapses that have inhibitory properties after hearing onset have excitatory qualities between MNTB neurons and LSO neurons during refinement. This is due to the fact that the K-Cl cotransporter KCC2, which is responsible for $Cl^-$ influx through GABA and glycine receptors, is not yet active [2]. The electrical gradient drives $Cl^-$ outward, causing the GABA or glycine neurotransmitters to trigger depolarization. Concomitantly, these synapses release glutamate during the first postnatal days [9]. Postsynaptic depolarization may cause $Ca^{2+}$ influx via $Ca_v^{2+}$ channels. $Ca^{2+}$ may act as an essential signal molecule in the process of refinement. To verify this hypothesis, and at the same time test if $Ca_v1.3$ plays a critical role in the process, we studied the process of refinement in the MNTB–LSO connection in *Cacna1d*$^{-/-}$ mice. As expected, refinement did not occur [13]. At present, we are still unable to rule out that the absence of refinement may, at least in part, be due to a lack of spontaneous activity in the auditory pathway. Preliminary results suggest that, in LSO neurons, $Ca_v1.3$-mediated local $Ca^{2+}$ influx represents a signal that is essential for the refinement of synaptic connections.

## Loss of $Ca_v1.3$ results in altered processing of acoustic signals

Loss of peripheral neuronal activity in sensory systems may cause severe anatomical and functional changes in the processing centers of the central nervous system [19]. Initially, it was unclear to what extent the numerous central auditory changes are attributable to local loss of $Ca_v1.3$, i.e., in auditory nuclei, or a lack of activity in the auditory nerve due to missing excitation transmission from cochlear IHC. Answering this question has significant biomedical implications. Cochlear implants can partially compensate for peripheral hearing loss, not, however, for central nervous system deficiencies. If mutations in so-called deafness genes affect not only the inner ear but also the auditory pathway, this may be the reason why some patients do not derive any benefit from cochlear implants [32]. To characterize local $Ca_v1.3$ function in the auditory path-

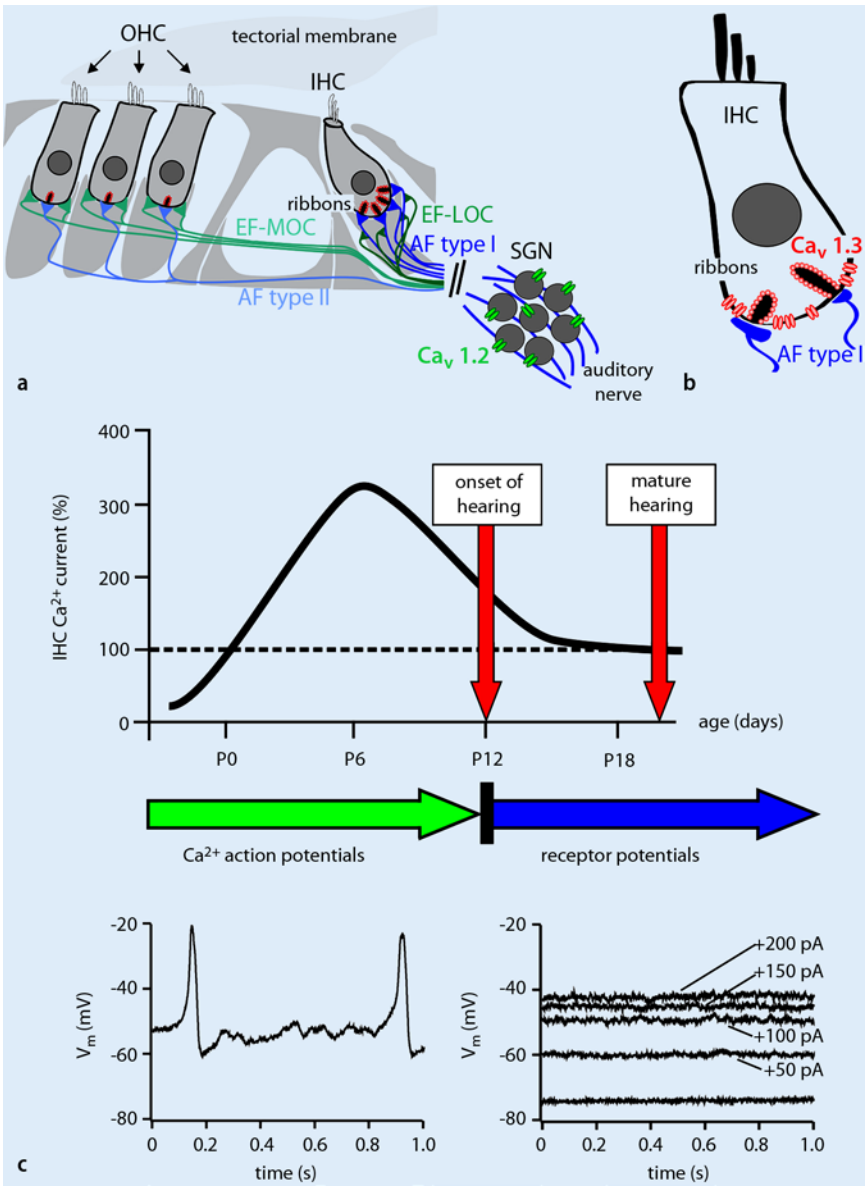


**Fig. 1** ▲ $Ca_v1.3$ and $Ca_v1.2$ channels in the cochlea. **a** Schematic illustration of the mature organ of Corti: cross section with three rows of outer (*OHC*) and one row of inner hair cells (*IHC*) and the tectorial membrane located above. Each IHC is innervated by several (10–20) type I afferent fibers (*AF type I*), whereas OHC are convergently innervated by a small number of type II afferents. The auditory nerve consists of the axons of the primary auditory spiral ganglion neurons (*SGN*). The SGN somata contain $Ca_v1.2$ channels. Efferents of the lateral olivocochlear bundle (*EF-LOC*) innervate type I afferents, whereas efferents of the medial olivocochlear bundle (*EF-MOC*) make direct contact with OHCs. **b** IHC with basolateral presynaptic specializations (*ribbons*) and adjacent presynaptic $Ca_v1.3$ channels. Each type I afferent connects to an active zone, i.e., to a ribbon with synaptic vesicles. **c** Course of development of murine $Ca_v1.3$ currents, *P0* = day of birth. $Ca_v1.3$ amplitude peaks at day 6 and is downregulated to reach a steady, lower value at day 15. (According to [16]). Between P0 and P12, IHC generate spontaneous $Ca^{2+}$ action potentials (*bottom left*), whereas, from P12, they produce graded receptor potentials rather than action potentials, even if current injection strength varies (*bottom right*)

way, we used the Cre-*loxP* system to generate site-specific excision of the channel isoform in the auditory brain stem. To this end, we made use of a smart conditional *Cacna1d-eGFP(flex)* allele. In this mouse strain, *loxP*-mediated deletion of an essential part of the *Cacna1d* gene causes the enhanced green fluorescent protein (eGFP) gene to be expressed [26]. By using an *Egr2:: Cre* driver line, we were able to perform targeted ablation of the gene in large areas of the auditory brain stem at an early embryonic stage, while the inner ear and auditory cortex remained unaffected.

Subsequent examination of these mice ($Cacna1d^{Egr2}$) revealed, as in $Cacna1d^{-/-}$ mice, a dramatic volume reduction of auditory nuclei due to the loss of numerous neurons (**Fig. 2c**, [26]). It was interesting to note that the inner ear of these region-specific knockout mice remained fully intact, which enabled us to measure so-called auditory brain stem responses. To this end, anesthetized mice were exposed to sound, while excitation along the auditory pathway was recorded using electrodes attached to the cranium, as is done in an electroencephalogram, with single peaks corresponding to the activity of specific regions of the auditory pathway (**Fig. 2e**). This analysis demonstrated that brain stem potentials in $Cacna1d^{Egr2}$ mice are drastically modified (**Fig. 2f**). In particular, potentials generated by the cochlear nuclei and the superior olivary complex, the first two structures along the central auditory pathway, showed significantly increased amplitudes in $Cacna1d^{Egr2}$ mice [26]. Hence, also locally, $Ca_v1.3$ plays an essential role for auditory pathway development and function. These findings have biomedical implications as there are deaf patients carrying mutated *Cacna1d* genes (cf. "The multifunctional $Ca_v1.3$ L-type $Ca^{2+}$ channel in the inner ear"). If the results gained from mice also apply to humans—which we do not doubt—cochlear implants would be of limited use in restoring hearing in these patients as they are highly likely to exhibit tremendous anatomical and functional deficiencies in the auditory brain stem. Interestingly, a review of the literature revealed retrocochlear function of other peripheral deafness genes in the auditory pathway as well [32]. Detailed analysis of functional central auditory deficiencies in mutations of these genes will provide more reliable predictions of potential benefit from cochlear implantation, indicating if central nervous system implants should be used in such cases.

## $Ca_v1.2$ and hearing damage

Intense noise causes irreversible damage to the cochlea, and, due to the loss of OHCs, threshold elevation (**Fig. 1a**). By

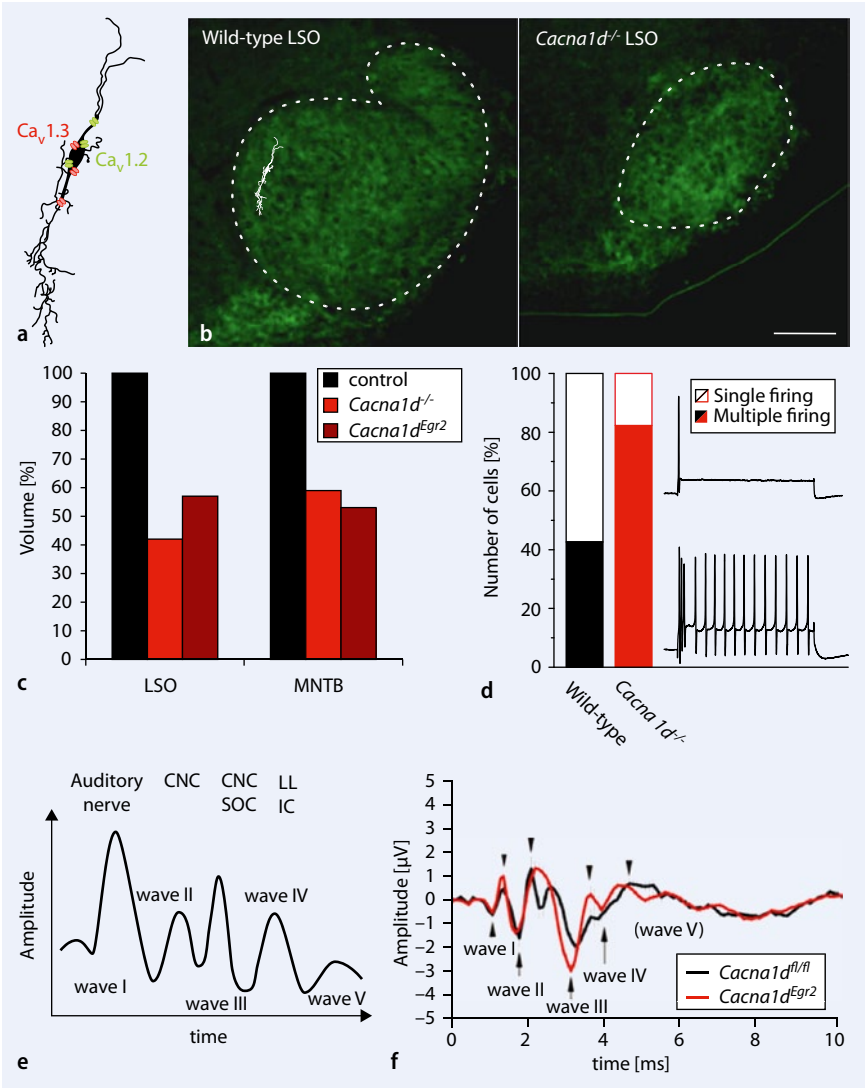


**Fig. 2** ▲ $Ca_v1.3$ and $Ca_v1.2$ channels in the auditory brain stem. **a** Schematic illustration of a lateral superior olive (*LSO*) neuron, expressing $Ca_v1.3$ and $Ca_v1.2$ channels in the soma and dendrites. **b** Immunohistochemical analysis of VGlut1 demonstrates an abnormal LSO in *Cacna1d*$^{Erg2}$ mice. The *white* neuron indicates the position of the neuron shown in **a**. **c** Drastic volume reduction of auditory nuclei in constitutive *Cacna1d*$^{-/-}$ mice or conditional *Cacna1d*$^{Erg2}$ mice. **d** Altered firing properties of LSO neurons in *Cacna1d*$^{Erg2}$ mice. *Solid bars* represent neurons with multiple firing pattern. *Open bars* represent neurons with single firing pattern. On the *right* are examples of neurons with single or multiple AP firing pattern. **e** Contribution of auditory structures to the waves of the auditory brain stem response. **f** Altered auditory brain stem responses in *Cacna1d*$^{Erg2}$ mice. Data from wild types are shown in *black*, from constitutive or conditional mice in *red*. *LSO* lateral superior olive, *MNTB* medial nucleus of the trapezoid body, *CNC* cochlear nuclear complex, *SOC* superior olivary complex, *LL* lateral lemniscus, *IC* inferior colliculus

contrast, moderate noise does not immediately result in measurable hearing loss, although, over time, growing loss of presynaptic IHC ribbons or loss of auditory nerve fibers connecting to them (**Fig. 1a**) are manifest [14, 22, 25]. Both phenomena exacerbate with age, and are associated, for instance, with impaired speech intelligibility. Studies of the inner ear revealed expression of $Ca_v1.2$ in the auditory nerve and inhibitory efferents projecting to the OHCs [11, 31].

In the central nervous system, $Ca_v1.2$-mediated $Ca^{2+}$ influx may, among other processes, be implicated in the expression of brain-derived neurotrophic factor (BNDF). Recently, BDNF was shown to have a protective as well as damaging effect on the somatosensory system [4] and the peripheral auditory system [33].

As systemic deletion of $Ca_v1.2$ causes embryonic death in mice [27], the role of $Ca_v1.2$ for the auditory system was examined using conditional mouse models. To this end, mice in which $Ca_v1.2$ was deleted, using Cre-*LoxP* either in the cochlea or in auditory centers implicated in efferent feedback from the brain stem, were compared. After acoustic trauma, *Cacna1c*$^{Egr2}$ mice with specific deletion in auditory brain stem nuclei exhibited the same amount of threshold elevation as controls. In *Cacna1c*$^{Pax2}$ mice with specific deletion of the $Ca_v1.2$ channel isoform in the cochlea, however, we observed less threshold elevation than in the corresponding controls, revealing that this channel in cochlear neurons is instrumental in the damaging effect caused by noise-induced trauma. This evidence points to the fact that, by analogy with the somatosensory system, for which a function in pain perception through BDNF upregulation is being discussed [7, 8, 30], BDNF also mediates damage in the cochlea during noise-induced trauma.

## Conclusions and recommendations

**All previous analyses have revealed an essential role of $Ca_v1.3$ for the inner ear and the central auditory pathway. *Cacna1d* therefore represents a prototypal deafness gene, mutations of which affect the inner ear as well as the auditory pathway. It remains unclear which signaling cascades are used by $Ca_v1.3$ to contribute to the survival of auditory nuclei, and whether the channel is locally required for the refinement of inhibitory neurons. Moreover, $Ca_v1.2$ also seems to play a vital role in the auditory system. It would therefore be interesting to further research its role to find out to what extent the functions of these two closely similar isoforms overlap or differ.**

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Marlies Knipper did her studies and her dissertation in biology at the University of Osnabrück. Subsequently, she completed postdoctoral research in the field of physiology at the University of Stuttgart-Hohenheim and at the University of London Ontario (Canada). In 1993, she became project leader of the AG Molecular Neurobiology at the Tübingen Hearing Research Center (THRC) and in 1996 she completed her habilitation. In 2008, she became professor for molecular physiology of hearing at the University of Tübingen. Since 2009, she has been a member of the National Academy of Sciences, Leopoldina, and since 2011 the deputy speaker of the Center for Neurosensory Science (ZfN) at the University of Tübingen.

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Eckhard Friauf studied biology at the University of Marburg. After his diploma (1992), he obtained a DAAD stipend to spend 1 year at New York University Medical Center in Manhattan. He completed his PhD in 1987 in Tübingen under the guidance of Hans Ulrich Schnitzler. After a postdoctoral stay with Carla Shatz at Stanford University, he returned to Tübingen, where he completed his habilitation in animal physiology in 1994. From 1995 to 1999, he held a C3 professorship for physiology and neurobiology at the University of Frankfurt. There, he was speaker of the graduate school "Neuronal plasticity: molecules, structures, function." In 1999, he moved to a C4 professorship in animal physiology at the University of Kaiserslautern. From 2000 to 2006, he served on the DFG board for graduate schools, and since 2012 he has been a member of the DFG Review Board "Neurosciences." In the same year, he also became coordinator of the DFG Priority Program 1608 "Ultrafast and temporally precise information processing: normal and dysfunctional hearing." His awards include: Attempto Prize of the University of Tübingen 1986; Landesakademiepreis Rheinland-Pfalz 2005.

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## Compliance with ethical guidelines

**Conflict of interest.** H.G. Nothwang, J. Engel, M. Knipper, and E. Friauf state that there are no conflicts of interest. All national guidelines on the care and use of laboratory animals have been followed and the necessary approval was obtained from the relevant authorities.

## References

1. Baig SM, Koschak A, Lieb A et al (2010) Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. Nat Neurosci 14:77–84
2. Balakrishnan V, Becker M, Loehrke S et al (2003) Expression and function of chloride transporters during development of inhibitory neurotransmission in the auditory brainstem. J Neurosci 23:4134–4145
3. Beutner D, Moser T (2001) The presynaptic function of mouse cochlear inner hair cells during development of hearing. J Neurosci 21:4593–4599
4. Biggs JE, Lu B van, Stebbing MJ et al (2010) Is BDNF sufficient for information transfer between microglia and dorsal horn neurons during the onset of central sensitization? Mol Pain 6:44
5. Brandt A, Striessnig J, Moser T (2003) CaV1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. J Neurosci 23:10832–10840
6. Clause A, Kim G, Sonntag M et al (2014) The precise temporal pattern of prehearing spontaneous activity is necessary for tonotopic map refinement. Neuron 82:822–835
7. Coull JA, Beggs S, Boudreau D et al (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438:1017–1021
8. Favereaux A, Thoumine O, Bouali-Benazzouz R et al (2011) Bidirectional integrative regulation of Cav1.2 calcium channel by microRNA miR-103: role in pain. EMBO J 30:3830–3841
9. Gillespie DC, Kim G, Kandler K (2005) Inhibitory synapses in the developing auditory system are glutamatergic. Nat Neurosci 8:332–338
10. Glowatzki E, Fuchs PA (2000) Cholinergic synaptic inhibition of inner hair cells in the neonatal mammalian cochlea. Science 288:2366–2368
11. Green GE, Khan KM, Beisel DW et al (1996) Calcium channel subunits in the mouse cochlea. J Neurochem 67:37–45
12. Hirtz JJ, Boesen M, Braun N et al (2011) Cav1.3 calcium channels are required for normal development of the auditory brainstem. J Neurosci 31:8280–8294
13. Hirtz JJ, Braun N, Griesemer D et al (2012) Synaptic refinement of an inhibitory topographic map in the auditory brainstem requires functional CaV1.3 calcium channels. J Neurosci 32:14602–14616
14. Jaumann M et al (2012) cGMP-Prkg1 signaling and Pde5 inhibition shelter cochlear hair cells and hearing function. Nat Med 18:252–259
15. Johnson SL, Kuhn S, Franz C et al (2013) Presynaptic maturation in auditory hair cells requires a critical period of sensory-independent spiking activity. Proc Natl Acad Sci U S A 110:8720–8725
16. Johnson SL, Marcotti W, Kros CJ (2005) Increase in efficiency and reduction in Ca2+ dependence of exocytosis during development of mouse inner hair cells. J Physiol 563:177–191

17. Jurkovicova-Tarabova B, Griesemer D, Pirone A et al (2012) Repertoire of high voltage-activated calcium channels in lateral superior olive: functional analysis in wild-type, Cav1.3−/−, and Cav1.2DHP−/− mice. J Neurophysiol 108:365–379
18. Kandler K (2004) Activity-dependent organization of inhibitory circuits: lessons from the auditory system. Curr Opin Neurobiol 14:96–104
19. Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138
20. Kim G, Kandler K (2003) Elimination and strengthening of glycinergic/GABAergic connections during tonotopic map formation. Nat Neurosci 6:282–290
21. Kros CJ, Ruppersberg JP, Rusch A (1998) Expression of a potassium current in inner hair cells during development of hearing in mice. Nature 394:281–284
22. Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29:14077–14085
23. Lohmann C, Ilic V, Friauf E (1998) Development of a topographically organized auditory network in slice culture is calcium dependent. J Neurobiol 34:97–112
24. Platzer J, Engel J, Schrott-Fischer A et al (2000) Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca2+ channels. Cell 102:89–97
25. Ruttiger L, Singer W, Panford-Walsh R et al (2013) The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. PLoS One 8:e57247
26. Satheesh SV, Kunert K, Ruttiger L et al (2012) Retrocochlear function of the peripheral deafness gene Cacna1d. Hum Mol Genet 21:3896–3909
27. Seisenberger C, Specht V, Welling A et al (2000) Functional embryonic cardiomyocytes after disruption of the L-type alpha1 C (Cav1.2) calcium channel gene in the mouse. J Biol Chem 275:39193–39199
28. Simmons DD (2002) Development of the inner ear efferent system across vertebrate species. J Neurobiol 53:228–250
29. Sinnegger-Brauns MJ, Hetzenauer A, Huber IG et al (2004) Isoform-specific regulation of mood behavior and pancreatic beta cell and cardiovascular function by L-type Ca 2+ channels. J Clin Invest 113(10):1430–1439
30. Trang T, Beggs S, Salter MW (2011) Brain-derived neurotrophic factor from microglia: a molecular substrate for neuropathic pain. Neuron Glia Biol 7:99–108
31. Waka N, Knipper M, Engel J (2003) Localization of the calcium channel subunits Cav1.2 (alpha1C) and Cav2.3 (alpha1E) in the mouse organ of Corti. Histol Histopathol 18:1115–1123
32. Willaredt MA, Ebbers L, Nothwang HG (2014) Central auditory function of deafness genes. Hear Res 312C:9–20
33. Zuccotti A, Kuhn S, Johnson SL et al (2012) Lack of brain-derived neurotrophic factor hampers inner hair cell synapse physiology, but protects against noise-induced hearing loss. J Neurosci 32:8545–8553