Published in final edited form as:

Hear Res. 2017 March ; 345: 57–68. doi:10.1016/j.heares.2017.01.001.

Genetic Perturbations Suggest a Role of the Resting Potential in Regulating the Expression of the Ion Channels of the KCNA and HCN families in Octopus Cells of the Ventral Cochlear Nucleus

Xiao-Jie Cao and Donata Oertel

Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53705

Abstract

Low-voltage-activated K^+ (g_{KL}) and hyperpolarization-activated mixed cation conductances (g_h) mediate currents, I_{KL} and I_h , through channels of the Kv1 (KCNA) and HCN families respectively and give auditory neurons the temporal precision required for signaling information about the onset, fine structure, and time of arrival of sounds. Being partially activated at rest, g_{KL} and g_h contribute to the resting potential and shape responses to even small subthreshold synaptic currents. Resting g_{KL} and g_h also affect the coupling of somatic depolarization with the generation of action potentials. To learn how these important conductances are regulated we have investigated how genetic perturbations affect their expression in octopus cells of the ventral cochlear nucleus (VCN). We report five new findings: First, the magnitude of g_h and g_{KL} varied over more than two-fold between wild type strains of mice. Second, average resting potentials are not different in different strains of mice even in the face of large differences in average g_{KL} and g_h . Third, I_{KL} has two components, one being α -dendrotoxin (α -DTX)-sensitive and partially inactivating and the other being α -DTX-insensitive, tetraethylammonium (TEA)-sensitive, and non-inactivating. Fourth, the loss of Kv1.1 results in diminution of the α -DTX-sensitive I_{KL} , and compensatory increased expression of an α -DTX-insensitive, tetraethylammonium (TEA)-sensitive I_{KL} . Fifth, I_h and I_{KL} are balanced at the resting potential in all wild type and mutant octopus cells even when resting potentials vary in individual cells over nearly 10 mV, indicating that the resting potential influences the expression of g_h and g_{KL} . The independence of resting potentials on g_{KL} and g_h shows that g_{KL} and g_h do not, over days or weeks, determine the resting potential but rather that the resting potential plays a role in regulating the magnitude of either or both g_{KL} and g_h .

Keywords

Homeostasis; genetic compensation; voltage-gated ion channels; KCNA; HCN; ventral cochlear nucleus

Corresponding author: Donata Oertel, Dept of Neuroscience, WIMR Room 5505, 1111 Highland Avenue, Madison, WI 53705-2275, doertel@wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Acoustic information is carried in the timing as well as the rate of firing of brain stem auditory neurons. The interplay between depolarization-activated, low-voltage-activated K^+ conductances (g_{KL}) mediated by ion channels of the Kv1 family and hyperpolarization-activated conductances (g_h) mediated through HCN channels gives auditory neurons the ability to receive and convey precisely timed electrical signals, as has been demonstrated in spiral ganglion cells (Mo and Davis, 1997; Liu et al., 2014; Mo et al., 2002; Kim and Holt, 2013), bushy and octopus cells in the VCN (Oertel, 1983; Manis and Marx, 1991; Rusznak et al., 1996; Cao et al., 2007), neurons in the medial nucleus of the trapezoid body (Brew and Forsythe, 1995; Cuttle et al., 2001), medial superior olive (Scott et al., 2005; Khurana et al., 2011), lateral superior olive (Barnes-Davies et al., 2004), and the ventral nucleus of the lateral lemniscus (Wu, 1999; Berger et al., 2014). In octopus cells g_{KL} and g_h are especially large (Bal and Oertel, 2000; Bal and Oertel, 2001; Ferragamo and Oertel, 2002; Cao and Oertel, 2011; McGinley et al., 2012). These conductances have opposite voltage dependence of activation and at rest the currents flow in opposite directions, I_{KL} flowing outward and I_h flowing inward, so that perturbations of the resting potential in either direction draw the voltage back toward rest.

The presence of g_h and g_{KL} enhances the timing of signaling in neurons in three ways. First, the partial activation of both conductances lowers the resting input resistance and speeds the rise and fall of voltage changes. Second, the voltage sensitivity around rest and the rapidity of the activation of g_{KL} enables g_{KL} to shorten and sharpen synaptic potentials. Third, the rapidity of the activation of g_{KL} makes the firing of neurons that have this conductance sensitive to the rate at which they are depolarized, and thus makes them effective coincidence detectors. Since the rate of depolarization depends on the temporal scatter of coincident subthreshold inputs, the sensitivity to the rate of depolarization sharpens the window over which octopus cells detect coincident firing in their auditory nerve inputs (Ferragamo and Oertel, 2002; McGinley and Oertel, 2006; Golding and Oertel, 2012).

g_{KL} is mediated through tetrameric, voltage-sensitive K^+ channels of the Kv1 family; four α subunits associate with four β subunits that modulate functional properties. Kv1.1 α subunits are present on the somatic and dendritic membranes of octopus cells as well as at perinodes of auditory nerve axons and octopus cell axons (Oertel et al., 2008; Rusznak et al., 2008; Robbins and Tempel, 2012). Immunohistochemical visualization of Kv1.1 (Oertel et al., 2008; Robbins and Tempel, 2012), Kv1.2 (Rusznak et al., 2008), Kv1.4 (Fonseca et al., 1998; Lujan et al., 2003), and Kv1.6 (Rusznak et al., 2008) subunits suggests that these subunits are expressed in octopus cells. The sensitivity of g_{KL} to DTX K, a blocker that is specific for Kv1.1 containing channels (Robertson et al., 1996), confirms the role of Kv1.1 in g_{KL} (Bal and Oertel, 2001). To be expressed at the membrane, Kv1.1 subunits require coassembly with other subunits, most often with Kv1.2 and Kv1.4 (Hopkins et al., 1994; Manganas and Trimmer, 2000; Ovsepian et al., 2016). The sensitivity of g_{KL} to α -DTX and to tityustoxin, selective for channels containing Kv1.2 subunits (Werkman et al., 1993; Robertson et al., 1996; Owen et al., 1997; Wang et al., 1999b; Wang et al., 1999a; Hopkins, 1998), functionally confirms the role of Kv1.2 subunits in octopus cells (Bal and Oertel, 2001).

g_h is mediated through tetrameric voltage-sensitive channels that are composed of HCN1-4 α subunits (Ludwig et al., 1998; Robinson and Siegelbaum, 2003). HCN1, HCN2 and HCN4 are expressed in the VCN (Moosmang et al. 1999; Koch et al. 2004; Notomi and Shigemoto 2004) and HCN1 and HCN2 have been shown specifically to be expressed in octopus cells (Koch et al., 2004). Homomeric HCN1 channels have the most rapid kinetics, HCN2 channels are slower, and HCN4 are the slowest; heteromeric channels have intermediate properties (Santoro et al., 1998; Moosmang et al., 2001; Ulens and Tytgat, 2001; Altomare et al., 2003; Whitaker et al., 2007). ZD7288 blocks g_h in many neurons, including in octopus cells (Harris and Constanti, 1995; Khakh and Henderson, 1998; Luthi and McCormick, 1998; Maccaferri and McBain, 1996; Bal and Oertel, 2000). In spiral ganglion cells g_h is formed from HCN1, HCN2, and HCN4 subunits (Kim and Holt, 2013; Liu et al., 2014). Many other auditory neurons also express g_h (Banks et al., 1993; Brew and Forsythe, 1995; Rusznak et al., 1996; Wu, 1999; Cuttle et al., 2001; Dodson et al., 2002; Mo et al., 2002; Svirskis et al., 2004; Cao et al., 2007; Khurana et al., 2011). The HCN channels that mediate I_h are permeable to both Na^+ and K^+ and have a reversal potential of -40 mV so that I_h is inward at the resting potential in octopus and many other types of neurons (Banks et al., 1993; Bal and Oertel, 2000).

HCN1 and Kv1.1 are colocalized in the somatic and dendritic octopus cell membrane (Oertel et al., 2008; Rusznak et al., 2008; Robbins and Tempel, 2012). In an earlier study we examined mice in which HCN1 was eliminated to alter g_h (Cao and Oertel, 2011). We found that in octopus cells of HCN1 null mutants g_{KL} is concomitantly reduced with g_h leading us to conclude that g_h governs g_{KL} (Cao and Oertel, 2011). Here we test the generality of that conclusion by addressing the complementary question, whether removing Kv1.1, a subunit that is present in many g_{KL} channels, alters I_{KL} or I_h or both. The present results support the earlier conclusion that g_h governs the expression of g_{KL} because we find that g_{KL} in Kv1.1 null mutants at the steady state is unchanged, that the loss of Kv1.1 is compensated by other subunits.

The large size of g_h and g_{KL} in octopus cells allows us to resolve and compare the expression of these conductances at the resting potential in different strains of mice. We found that at the resting potential, the magnitude of I_h equals the magnitude of the sum of an α -DTX-sensitive and an α -DTX-insensitive, TEA-sensitive I_{KL} in each of five strains of mutant and wild type mice even when resting potentials varied between cells over nearly 10 mV and g_h varied over a five-fold range. It has been suggested by many investigators that g_h and g_{KL} set the resting potential. That is true in the short term but the present experiments show that in the long term it is the resting potential that regulates the g_{KL} so that I_{KL} balances I_h at rest.

2. Materials and Methods

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the School of Medicine and Public Health at the University of Wisconsin-Madison (M005303).

2.1 Mice

Bruce Tempel kindly sent us mice that lack the Kv1.1 subunit (Smart et al., 1998). The null mutant was generated by injecting AB-1 embryonic stem cells that contained a *Kcna1* targeting vector into C57BL/6J blastocysts. Chimeric mice that expressed the null mutation in the germline were then bred to C3HeB/FeJ mice and have been inbred for more than 15 generations. The absence of Kv1.1 leads to seizures and neuromyotonia and to enhanced excitability at neuromuscular junctions at high temperatures (Smart et al., 1998; Zhou et al., 1998). The mouse line was maintained by breeding heterozygote mice. Genotyping was based on tail digests with three primers, a wild-type forward, a mutant forward and a common reverse, as outlined on the Tempel website (<http://depts.washington.edu/tempelab/Protocols/KCNA1.html>). Alkaline digests were used to obtain DNA from tails. DNA was then combined with nucleotides, primers and Flexi-Go Taq DNA polymerase, amplified over 30 PCR cycles, and run on gels. Measurements in homozygous Kv1.1 null mice were compared with measurements in wild type control mice.

Mice that lack the HCN1 subunit were genetically engineered by Nolan et al. (2003); the mice used for the present experiments were propagated by inbreeding homozygous mutants from a breeding pair purchased from the Jackson Laboratory (ME) (Cao and Oertel, 2011). Experiments were performed using HCN1^{-/-} mice constructed from 129S/SvEv-derived MM13 embryonic stem cells that were injected into and crossed back into C57BL/6 for more than 10 generations. To avoid genetic drift, all measurements on HCN1 null mice were made within two years of their having been purchased from Jackson Labs. For these mice C57BL/6 mice served as wild type controls.

ICR mice are outbred mice that we purchase as needed from what was formerly Harlan (Madison, Wisconsin), and is now Envigo. We generally receive a mother with a litter of mice of a specified age range.

2.2 Solutions

All chemicals were from Sigma-Aldrich, unless stated otherwise.

Normal saline was used for dissections and for current clamp measurements: The normal extracellular solution in which slices were cut and maintained comprised (in mM) 130 NaCl, 3 KCl, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgSO₄, 20 NaHCO₃, 6 HEPES, 10 glucose, and 0.4 ascorbic acid saturated with 95% O₂-5% CO₂, pH 7.3–7.4, between 24 and 33 °C. The osmolality was 306 mOsm/kg (3D3 Osmometer, Advanced Instruments Inc, Norwood, MA).

Phosphate was eliminated from control saline solution for voltage-clamp measurements of I_h and I_{KL} contained (in mM): 138 NaCl; 4.2 KCl, 2.4 CaCl₂, 1.3 MgCl₂, 10 HEPES, 10 glucose, 40 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Tocris Cookson, UK), 1 μM strychnine, pH 7.4 and saturated with 100% O₂. In voltage-clamp experiments to measure I_h or I_{KL}, the voltage-sensitive sodium current was blocked by 1 μM tetrodotoxin (TTX), the voltage-sensitive calcium current was blocked by 0.25 mM CdCl₂. Measurements of I_h were made in the additional presence of 50 nM α-dendrotoxin (α-DTX) to block the α-DTX-sensitive component of I_{KL} (Bal and Oertel 2000). The α-DTX-insensitive I_{KL}, about 0.1 nA

(Fig. 5), remained but comprises a small proportion of measured I_h over most of the voltage-range. Measurements of I_{KL} were made in the presence of 50 μ M ZD7288 to block I_h .

Recording pipettes were filled with a solution that consisted of (in mM): 108 potassium gluconate, 9 HEPES, 9 EGTA, 4.5 $MgCl_2$, 14 phosphocreatine (tris salt), 4 ATP (Na salt) and 0.3 GTP (tris salt). The pH was adjusted to 7.4 with KOH; the osmolality was 303 mOsm/kg.

2.3 Preparation of slices

Coronal slices of the most caudal part of the VCN were made from mice between 16 and 22 days after birth. Slices, 210 μ m thick, were cut with a vibrating microtome (Leica VT 1000S). Slices were maintained in a recording chamber (~0.6 ml) that was superfused with normal saline at 5 to 6 ml/min. The recording chamber was mounted on the stage of a compound microscope (Zeiss Axioskop) and viewed through a 63X water immersion objective whose image was displayed through a Hamamatsu CCD camera (C2400-77AH) on a video monitor. The temperature was measured between the inflow of the chamber and the tissue with a Thermalert thermometer (Physitemp) through a small thermistor (IT-23, Physitemp, diameter: 0.1 mm). The output of the Thermalert thermometer was fed into a custom-made, feedback-controlled heater that heated the saline in glass tubing (1.5 mm inner, 3 mm outer diameter) just before it reached the chamber to keep the temperature constant at 33°C. An adjustable delay in the controller for the heater prevented temperature oscillations. Recordings were generally made within two hours after slices were cut.

2.4 Electrophysiological recordings

Patch-clamp recordings were made with pipettes of borosilicate glass and had resistances that ranged between 4 and 8 M Ω . Recordings were made with an Axopatch 200A amplifier (Axon Instruments). Records were digitized at 50 kHz and low-pass filtered at 10 kHz. All reported results were from recordings in which ~90% of the series resistance could be compensated on-line with 10 μ s lag; no corrections were made for errors in voltage that resulted from uncompensated series resistance. Series resistances were between 10 and 14 M Ω in each of the cell types, of which ~90% could be compensated in octopus cells. Recordings in which series resistances were >14 M Ω , were disregarded. The output was digitized through a Digidata 1320A interface (Axon Instruments) and fed into a computer. Stimulation and recording was controlled by pClamp 9 software (Axon Instruments). Analyses were performed with Clampfit 9. Input resistances were measured as the slope of plots of voltage responses to small hyperpolarizing current steps. The slope was measured over the region ~10 mV from the resting potential. Statistical analyses were made with Origin software (version 7.5); the results are given as means $\pm$ standard deviation, with n being the number of cells in which the measurement was made.

All reported voltages were compensated for a -12 mV junction potential.

3. Results

3.1 Influence of g_h and g_{KL} on octopus cells

Octopus cells were identified by their electrophysiological characteristics in recordings in current clamp: their low input resistance, the small size of somatic action potentials, and by strong rectification in both the depolarizing and hyperpolarizing directions (Golding et al., 1995; Bal and Oertel, 2000) (Fig. 1A, left panel). One way to demonstrate the presence of g_{KL} is to test the cell's sensitivity to its blockers (Bal and Oertel, 2001). α -DTX, at 50 nM, blocks K^+ channels that have Kv1.1, Kv1.2 and Kv1.6 subunits (Hopkins et al., 1994; Hopkins, 1998; Harvey, 2001). In octopus cells it blocks a current that reverses at -77 mV when E_K is -85 mV under conditions identical to those used in the present study (Bal and Oertel, 2001). The application of 50 nM α -DTX broadened action potentials and evoked repetitive firing (Bal and Oertel, 2001; Ferragamo and Oertel, 2002) (Fig. 1A). The peak of the first action potential increased from 18 mV to 29 mV, showing that g_{KL} affects the electrical coupling of events in the soma and in the axon. Subsequent action potentials were smaller than the first. The larger size of voltage changes in responses to current pulses reflects an increase in input resistance. The application of 50 nM α -DTX also depolarized the resting potential of the octopus cell by 5.7 ± 0.9 mV ($n=3$) as reported previously (6.2 ± 2.5 mV, Bal and Oertel, 2001).

Similarly we can demonstrate the role of g_h by blocking it. The reversal potential of I_h was measured to be -40 mV under conditions identical to those used in the present study (Bal and Oertel, 2000). The application of 50 μ M ZD7288, a selective blocker of g_h in octopus cells (Harris and Constanti, 1995; Khakh and Henderson, 1998; Luthi and McCormick, 1998; Maccaferri and McBain, 1996; Bal and Oertel, 2000), removed the sag in voltage changes evoked by hyperpolarizing current pulses, increased the size of voltage changes, and slowed the rate of voltage changes in the hyperpolarizing voltage range (Fig. 1B). Action potentials evoked by depolarizing currents became slightly larger and wider; action potentials evoked by the offset of hyperpolarizing current pulses were attenuated, presumably because the slowing of voltage changes resulted in an increased temporal overlap in the activation of regenerative Na^+ currents and repolarizing K^+ currents. Blocking g_h also hyperpolarized the resting potential by 5 mV.

3.2 Resting potentials

The observation that blocking g_h or g_{KL} alters the resting potential would suggest that genetic manipulation of g_h or g_{KL} might alter the resting potentials. Unexpectedly we found that there is no significant difference in resting potentials between strains. A one-way ANOVA analysis showed that $p = 0.26$ so that at the 0.05 level there is no significant difference. We compare resting potentials in octopus cells in Figure 2 (some measurements were from recordings associated with earlier studies (Cao and Oertel, 2010, 2011)). Within each strain resting potentials varied between cells between -59 mV and -67 mV.

3.3 Measurements of g_{KL} and g_h under voltage-clamp

Unlike the application of blockers, which affect octopus cells over seconds and minutes, genetic perturbations affect the function of octopus cells throughout development over a

period of weeks. Octopus cells express Kv1.1, a subunit of ion channels that generate g_{KL} , and HCN1, a subunit of ion channels that generate g_h (Koch et al., 2004; Oertel et al., 2008; Rusznak et al., 2008; Robbins and Tempel, 2012). We examined octopus cells in which Kv1.1 was eliminated to alter g_{KL} and their wild type controls (Smart et al., 1998), and compared them with those in outbred ICR mice and in mice in which HCN1 was eliminated to alter g_h (Bal and Oertel, 2000; Bal and Oertel, 2001; Nolan et al., 2003; Cao and Oertel, 2011).

To isolate and measure I_{KL} , other voltage-sensitive currents were blocked pharmacologically: I_h was blocked by 50 μ M ZD7288, the voltage-dependent Na^+ current by 1 μ M tetrodotoxin (TTX), and the voltage-sensitive calcium current by 0.25 mM $CdCl_2$. Also, spontaneous excitatory and inhibitory synaptic inputs were blocked by 40 μ M DNQX and 1 μ M strychnine. As in ICR octopus cells (Bal and Oertel, 2001), the current-voltage relationship of the peak current begins to curve upward in mutant strains and their wild type controls at -70 mV as g_{KL} is activated; g_{KL} is, by definition, activated at relatively negative voltages (Fig. 3D). A high-voltage-activated K^+ conductance, g_{KH} , is activated at voltages from about -40 mV so that depolarizing voltage pulses to values more negative than -40 mV activate mainly I_{KL} (Manis and Marx, 1991; Brew and Forsythe, 1995; Rathouz and Trussell, 1998; Bal and Oertel, 2001). We use the size of those currents to make a rough comparison of the relative magnitudes of I_{KL} across strains (Fig. 3H). Depolarizing voltage steps from -90 mV to -40 mV evoked peak outward currents that decayed to a steady state level (Fig. 3A, black trace). In a Kv1.1 nullmutant octopus cell, the outward currents rose to only a barely perceptible peak but steady state levels were similar as in wild type controls (Fig. 3A, red trace). Averaged values from measurements in more cells show that there was a statistically significant difference in the peak levels but not in the steady state levels of I_{KL} between Kv1.1 null mutants and wild type controls (Fig. 3C). I_{KL} in Kv1.1 null mutants has a similar voltage-sensitivity as the wild type (Fig. 3D). HCN1 null mutants have outward currents that are smaller but whose inactivation follows a similar time course as wild type controls (Fig. 3B). The voltage-sensitivity of I_{KL} in HCN1 null mutants is similar as in wild type controls (Fig. 3D).

A parallel series of measurements was made of g_h . To separate I_h , other voltage-sensitive currents were blocked pharmacologically: I_{KL} was blocked by 50 nM α -DTX, the voltage-dependent Na^+ current by 1 μ M tetrodotoxin (TTX), and the voltage-sensitive calcium current by 0.25 mM $CdCl_2$; spontaneous excitatory and inhibitory synaptic inputs were blocked by 40 μ M DNQX and 1 μ M strychnine. In Kv1.1 null mutants, I_h was indistinguishable from the wild type (Fig. 3E, G). In HCN1 null mutants the maximum amplitude and activation rate of g_h were reduced relative to wild type controls (Fig. 3F) (Cao and Oertel, 2011). The voltage activation curve of g_h was similar in Kv1.1 null mutants as in wild type controls (Fig. 3G).

The magnitude of g_h and g_{KL} varied over a four-fold range even between wild type strains of mice (Fig. 3H) (Table 1). The present results extend previous findings that there are significant differences in the magnitudes of peak I_h and I_{KL} in different strains of mice and that I_h and I_{KL} covary ($R = 0.95$) (Cao and Oertel, 2011) (Fig. 3H). Maximal g_h and g_{KL} , derived from peak currents, are largest in the outbred albino ICR mice, 419 nS g_{KL} and 116

nS g_h , values that are comparable with those measured previously (Bal and Oertel, 2000; Bal and Oertel, 2001). Maximal g_h and g_{KL} were smaller in HCN1 wild type controls, F2 hybrids of 129S and C57Bl/6 inbred strains, 90 nS g_{KL} and 61 nS g_h . They are smallest in Kv1.1 wild type controls, C3HeB/FeJ, 60 nS g_{KL} and 55 nS g_h . Altering g_h by eliminating HCN1 resulted in a reduction of both g_h and g_{KL} by about 40% relative to the wild type controls (Cao and Oertel, 2011) whereas eliminating Kv1.1 reduced only the peak g_{KL} (Fig. 3C).

The sensitivity of I_{KL} in Kv1.1 null mutants to α -DTX differs from that of wild type controls (Fig. 4). In wild type controls, as in ICR mice (Bal and Oertel, 2001), α -DTX blocked 80% to 90% of the outward current that was activated at voltages more negative than -50 mV and it blocked most of the transient peak current. In Kv1.1 wild type control mice in the presence of α -DTX, the current-voltage relationship was linear to about -50 mV (Fig. 4A, right panel, dashed line), indicating that α -DTX blocked most of I_{KL} ; an α -DTX-insensitive component was activated at voltages more positive than -50 mV. In Kv1.1 null mutants, in contrast, an α -DTX-insensitive current was activated at -70 mV, a current that is by definition low-voltage-activated current. We tested the sensitivity of I_{KL} in wild type and Kv1.1 null mutant octopus cells to 100 nM DTX-I, a mamba snake toxin that has similar subunit selectivity as α -DTX (Harvey, 1997; Hopkins, 1998). It, like α -DTX, reduced the outward current but failed to block I_{KL} completely. These findings indicate that the subunit composition of channels that mediate I_{KL} differs in the Kv1.1 mutants and wild type. Unlike in wild type controls and other wild type strains, a substantial fraction of channels that mediate I_{KL} in Kv1.1 null mutant octopus cells do not contain Kv1.1 or Kv1.2 or Kv1.6 subunits.

3.4 Balance of I_h and I_{KL} at the resting potential

As g_h and g_{KL} are partially activated at rest we compared the relative magnitudes of the α -DTX-sensitive and the ZD7288-sensitive currents at the resting potential in five strains of mice, ICR, HCN1^{+/+}, HCN1^{-/-}, Kv1.1^{+/+}, and Kv1.1^{-/-}. To measure the relative magnitudes of those currents in individual octopus cells, the cell was held in voltage-clamp at its resting potential, the voltage at which there was no holding current (Oertel et al., 2000; Cao and Oertel, 2011). Subsequent application of 50 μ M ZD7288 to block I_h , left an unbalanced outward current equal to the magnitude of I_h (Bal and Oertel, 2000). Overall I_h at rest ranged over about a 4-fold range, 0.26 nA in an Kv1.1 null mouse to 1.1 nA in an ICR mouse (Table 2). Further application of 50 nM α -DTX to block I_{KL} in octopus cells blocked an outward current equal in magnitude to between 80 and 90% of the unbalanced outward current in four of the five strains. In the Kv1.1 null mutants, however, α -DTX blocked only about 50% of the outward current (Fig. 5). The α -DTX-insensitive current was larger not only in its proportion but also in absolute magnitude in Kv1.1 null mutant mice than in any of the other strains examined (Table 2). A one-way ANOVA to test the hypothesis that the values are similar resulted in $p < 0.0001$, indicating that the currents are not the same in all five strains.

In the two strains in which the α -DTX-insensitive component of I_{KL} is largest, ICR mice and Kv1.1 null mutant mice, we tested whether this component is sensitive to 10 mM TEA, a blocker of K^+ channels that has been demonstrated to block homomeric Kv1.1, Kv1.2,

Kv1.3, and Kv1.4 channels but not Kv1.5 channels (Stuhmer et al., 1989; Grissmer et al., 1994). In all ICR octopus cells ($n=4$) and Kv1.1 null cells ($n=4$) tested, 10 mM TEA blocked the α -DTX-insensitive component of the outward current (Fig. 6). In the other three strains the α -DTX-insensitive component of I_{KL} was too small to test reliably. We conclude that while in most strains, the α DTX-sensitive component of I_{KL} largely balances I_h , a population of α -DTX-insensitive channels that is less common in wild type animals compensates for the absence of Kv1.1-containing channels in Kv1.1 null mutants. A prerequisite to compensating for changes in I_{KL} is the need to detect I_{KL} . Our results suggest that the balance between steady state I_h and I_{KL} at rest may be the homeostatic feature that is regulated, that the sum of the α -DTX-sensitive and α -DTX-insensitive components I_{KL} are regulated to balance I_h at rest. We reach this conclusion because I_h and I_{KL} are balanced in every octopus cell even when the magnitudes of I_h and I_{KL} differ over a wide range and when the subunit composition of the ion channels that mediate these currents varies. This observation also indicates that I_h is the predominant inward current at rest in octopus cells (Fig. 6).

3.5 Rate-of-depolarization thresholds vary with peak g_{KL}

In octopus cells of Kv1.1 null mutants, outward currents evoked by depolarizing voltage pulses have reduced peaks but steady state currents are not different from their wild type controls (Fig. 3A). We therefore explored a function that depends mainly on the transient outward current, the rate-of-depolarization (dV/dt) threshold. The dV/dt threshold is a measure of the ability of neurons to convey the timing of their synaptic inputs with temporal precision (McGinley and Oertel, 2006).

The dV/dt thresholds are intrinsic properties of octopus cells, independent of the resting potential (McGinley and Oertel, 2006). Figure 7 illustrates how the rate-of-depolarization sensitivity was measured in a wild type and a mutant octopus cell. Cells were depolarized with currents that rose as ramps to a steady level (1 nA) with the duration of ramps varying systematically (Fig. 7C). This family of currents depolarized octopus cells at differing rates. Rapidly rising depolarizations evoked action potentials whereas slowly rising depolarizations did not (Fig. 7A, B). Plots of peak depolarization as a function of the slope of the depolarization are shown at the right. The step in the peak voltage occurs where the rate of depolarization goes from subthreshold to suprathreshold (Fig. 7A, B dashed lines). We define the rate-of-depolarization threshold (dV/dt threshold) as the slowest rate of depolarization that generates an action potential. Earlier studies have shown that the rate threshold measured with current protocols in which the maximal current instead of the duration of ramps are varied are similar (Ferragamo and Oertel, 2002; McGinley and Oertel, 2006). As the rate of depolarization increased, the peaks of action potentials became larger. Figure 7 shows that the wild type octopus cell required a faster depolarization (4.5 mV/ms) to reach threshold than did the Kv1.1 null mutant cell (2.8 mV/ms). The results of multiple such measurements show that octopus cells in Kv1.1 null mutants consistently have significantly lower rate-of-depolarization thresholds than wild type controls (Fig. 7D).

Because g_{KL} is reduced in amplitude but not kinetics in HCN1 null mutants relative to controls (Cao and Oertel, 2011), we also measured rate thresholds in those mice. The rate-

of-depolarization threshold was significantly reduced in octopus cells of HCN1 null mutant mice relative to HCN1 wild type controls. There was also a significant difference between wild type controls for Kv1.1 and HCN1 (Fig. 7D).

Octopus cells fire transiently in responses to depolarizing current pulses because the rapid activation of I_{KL} prevents later action potentials; when depolarizations are too slow, the activation of I_{KL} prevents firing altogether (Ferragamo and Oertel, 2002; McGinley and Oertel, 2006). We find that there is a strong correlation between estimates of peak I_{KL} at -40 mV and rate thresholds in octopus cells in different strains of mice ($R = 0.85$) (Fig. 7E). Octopus cells in HCN1 wild type controls had large I_{KL} peaks and also high dV/dt thresholds while octopus cells in Kv1.1 null mutant mice had a small I_{KL} and the lowest rate thresholds. Octopus cells in ICR mice have yet larger I_{KL} (18.5 nA at -40 mV, (Bal and Oertel, 2001)) and yet faster dV/dt thresholds (10.0 ± 2.5 mV/ms (Ferragamo and Oertel, 2002)).

The findings that dV/dt thresholds are reduced in the absence of Kv1.1 and HCN1 subunits indicate that these subunits, and the conductances of which they are a part, are important for conveying timing in acoustic information. They predict that Kv1.1 null and HCN1 null mice have deficits in facets of hearing that depend on detecting the fine structure and the onsets of sounds.

4. Discussion

In auditory neurons, the importance of interactions between g_h and g_{KL} for signaling has long been appreciated (Rothman and Manis, 2003b; Golding and Oertel, 2012). The relatively sluggish g_h helps give auditory neurons a low input resistance that makes voltage changes rapid while the speedy g_{KL} repolarizes not only suprathreshold but also subthreshold synaptic potentials (Oertel, 1983; Manis and Marx, 1991; Brew and Forsythe, 1995; Golding et al., 1995; Rathouz and Trussell, 1998; Dodson et al., 2002; Ferragamo and Oertel, 2002; Barnes-Davies et al., 2004; Rothman and Manis, 2003a; Brew et al., 2003; Kuba et al., 2005; Brew and Forsythe, 2005; Scott et al., 2005; Gittelmann and Tempel, 2006; McGinley and Oertel, 2006; Mathews et al., 2010; Karcz et al., 2011; Khurana et al., 2011; Golding and Oertel, 2012).

4.1 I_{KL} and I_h are balanced at the resting potential

Studies of g_h and g_{KL} are particularly revealing in octopus cells of the VCN because both conductances are expressed at high enough levels that their activation at the resting potential can be measured. A major finding of this study is that I_h and I_{KL} are balanced at the resting potential in all wild type and mutant octopus cells examined, even after the subunit composition of the ion channels that mediate those currents is perturbed genetically (Figs. 4 and 5). The current that was blocked by ZD7288, a blocker of HCN channels, was equal to the sum of an α -DTX-sensitive plus an α -DTX-insensitive but TEA-sensitive current. That balance was observed not only in wild type controls but also in HCN1 null mutants in which both g_h and g_{KL} were reduced and in Kv1.1 null mutants where only the peak g_{KL} was reduced. The consistency of this observation across mouse strains suggests that the balance of currents reflects direct regulation.

4.2 Homeostatic plasticity

Some of the rules that regulate the excitability of individual neurons in neuronal circuits have been elucidated (Golowasch et al., 1999b; Golowasch et al., 1999a; O'Leary et al., 2013; O'Leary et al., 2014). As in octopus cells, in many vertebrates and invertebrate neurons pairs or triplets of currents are correlated in amplitude, suggesting that they are coregulated (MacLean et al., 2003; Swensen and Bean, 2005; Vahasoyrinki et al., 2006; Bergquist et al., 2010; Huang et al., 2011; Zhao and Golowasch, 2012; Amendola et al., 2012; Golowasch, 2014). Coregulation of conductances is mediated through neuromodulators and also by electrical activity (Khorkova and Golowasch, 2007).

There has also been considerable interest in homeostatic plasticity, in the regulatory mechanisms that keep firing rates within their optimal range as some synapses are strengthened and others are weakened in the face of learning and remembering. Changes in firing rates of neurons that result from long-term potentiation or depression are followed by scaling of the strength of excitatory synapses or to change the number of synapses, to alter the balance of excitation and inhibition (Turrigiano, 1999; Turrigiano, 2007; Thiagarajan et al., 2002; Thiagarajan et al., 2005; Wheeler et al., 2008; Shepherd et al., 2006). Electrical excitability and electrical activity in a circuit depend not only on the strength of synaptic connections but also on the intrinsic electrical properties of the neurons upon which synapses act. These, too, are altered by electrical activity and chronic depolarization (O'Leary et al., 2010). How might a neuron detect electrical properties in order to regulate them? It has been suggested that intracellular Ca^{2+} levels might serve as error signals that generate changes in the mRNA and thus changes in expression levels of ion channels (O'Leary et al., 2014). It is possible that one role of voltage-gated Ca^{2+} channels in octopus cells is to monitor and regulate activity levels (Bal and Oertel, 2007). Because AMPA receptors of octopus cells are Ca^{2+} permeable, they would be expected to affect intracellular levels of Ca^{2+} as a function of synaptic input (Gardner et al., 1999, 2001; Cao and Oertel, 2010).

4.3 Homeostatic regulation of the resting membrane potential

How resting potentials of neurons are regulated and determined is not well understood and is inherently complicated because so many factors are involved, including electrogenic pumps, voltage-independent conductances, and voltage-sensitive conductances. One of the most extensive studies of the resting potentials of thalamocortical neurons concluded that the interplay of seven conductances controls the resting potential (Amarillo et al., 2014). Claims have been made about conductances, especially of g_h , that they are determinants of the resting potential but those claims are usually based on short-term changes in the resting potential after conductances are acutely blocked (Pape, 1996). Our results indicate that blocking g_h or g_{KL} acutely in octopus cells results in roughly a 5 mV change in the resting potential (Fig. 1). It is revealing that the genetic deletion of subunits of the ion channels that mediate these conductances nevertheless does not cause measurable changes of the average resting potentials of octopus cells (Fig. 2). These findings suggest that resting potentials are under homeostatic control and that acute changes in resting currents evoke slower compensatory changes. In principal cells of the medial nucleus of the trapezoid body, as in octopus cells, the deletion of Kv1.1 does not significantly alter the resting potential (Brew et

al., 2003; Brew et al., 2007). In neurons known to be depolarized by blocking the M-current, the deletion of KCNQ2 leaves the resting potentials of cortical neurons no different than in the wild type controls (Adams and Brown, 1982; Halliwell and Adams, 1982; Soh et al., 2014). On the other hand, unlike in octopus cells, spiral ganglion cells and hippocampal neurons in HCN1 null mice were hyperpolarized relative to wild type controls by similar amounts as by the application of ZD7288 (Huang et al., 2009; Kim and Holt, 2013). The mechanisms that regulate resting potentials thus differ between groups of neurons.

4.4 Compensation

Our results show that deletion of Kv1.1 results in the substitution of α -DTX-insensitive voltage-gated K^+ channels with slower kinetics for some of the α -DTX-sensitive K^+ channels. At the concentrations used, 50 nM α -DTX, this toxin blocks tetrameric Kv1 channels that contain Kv1.1, Kv1.2, and Kv1.6 (Hopkins et al., 1994; Hopkins, 1998; Harvey, 2001; Ovsepian et al., 2016). The sensitivity to α -DTX indicates that in wild type octopus cells 90% of g_{KL} is mediated through tetrameric channels that contain Kv1.1 and Kv1.2 and/or Kv1.6 subunits. Kv1.1 homomers are retained in the endoplasmic reticulum and are not expressed at the membrane thus most Kv1 channels in the plasma membrane that contain Kv1.1 subunits are heterotetramers that also contain Kv1.2 or Kv1.4 and possibly Kv1.6 subunits (Manganas and Trimmer, 2000; Rasband and Trimmer, 2001; Ovsepian et al., 2016). Kv1.1 subunits speed activation and shift the half-maximal activation voltage in the hyperpolarizing direction (Bagchi et al., 2014; Ovsepian et al., 2016).

What might the subunit composition be of the α -DTX-insensitive, TEA sensitive channels? They are unlikely to contain Kv1.3 because these subunits are expressed as heterotetramers with Kv1.2 and Kv1.6 subunits that would be expected to be sensitive to α -DTX (Ovsepian et al., 2016). One possibility is that those currents are generated from Kv1.4 homotetramers. There is some evidence that Kv1.4 subunits are expressed in octopus cells (Fonseca et al., 1998; Lujan et al., 2003). Homomeric channels would be expected to be insensitive to α -DTX, sensitive to TEA, and they are activated at low voltages (Stuhmer et al., 1989). Kv1.4 homotetrameric channels are reported to inactivate while in octopus cells of Kv1.1 null mutants, K^+ currents showed relatively little inactivation (Stuhmer et al., 1989; Ovsepian et al., 2016). However, inactivation was illustrated in those studies in expression systems and with responses to much larger depolarizations. Furthermore, expression systems are exempt from some forms of regulation and lacking some associated interacting proteins and so do not necessarily recapitulate the characteristics of native channels.

Levels of expression of Kv1 channels at the cell surface depend on how their trafficking from the Golgi apparatus to the plasma membrane is regulated. Trafficking to the axonal and terminal membranes of hippocampal neurons has been shown to be strongly influenced by Kv1.1 subunits (Manganas and Trimmer, 2000; Ovsepian et al., 2016). Heteromeric channels containing Kv1.1 or Kv1.4 were delivered to axonal membranes only with the inclusion of Kv1.2 (Jenkins et al., 2011). The β subunits also affect the function and trafficking of Kv1 channels (Manganas and Trimmer, 2000); KCNE4, for example, reduces currents through Kv1.1 and Kv1.3 α subunits (Grunnet et al., 2003). Glycosylation also affects the stability and trafficking Kv1 channels; all subunits except Kv1.6 have one or two

glycosylation sites. The channels are assembled in the Golgi apparatus where they are glycosylated at asparagine residues with N-glycans and further processed. When the glycosylation sites were removed, surface expression of Kv1.2, Kv1.3 and Kv1.4 decreased but expression of Kv1.1 was unaffected (Watanabe et al., 2015; Watanabe et al., 2007; Watanabe et al., 2004; Thayer et al., 2016). In hippocampal pyramidal cells, DTX-sensitive currents mediated by channels that contain Kv1.2 are also regulated by electrical activity through Ca^{2+} entry (Hyun et al., 2013). It has also been reported that cerebellar basket cells of heterozygotes that carry a mutation in Kv1.1, *Kcna1*^{V408A/+}, have normal somatic action potentials but broadened action potentials at terminals that resulted in an increased release of GABA that was not compensated (Begum et al., 2016).

In contrast to compensation for the loss of Kv1.1 subunits that involves substitution of channels with a differing subunit composition, compensation for the loss of HCN1 subunits was by the reduction of the expression of g_{KL} without a change in sensitivity to ZD7288. Presumably g_{h} in HCN1 null mutant octopus cells is formed from HCN2 and HCN4 subunits, subunits known to be expressed in auditory neurons (Bal and Oertel, 2000; Koch et al., 2004; Kim and Holt, 2013).

4.5 Expression of g_{h} and g_{KL} is linked by the resting potential

Two lines of evidence suggest that the regulation of g_{h} and g_{KL} is linked in octopus cells. First, the magnitudes of g_{h} and g_{KL} in different strains and different neurons in the VCN covary (Fig. 3H) (Cao and Oertel, 2011). The maximal conductances vary widely not only in the mutants but also among wild type strains and there is a positive relationship between maximal amplitudes of g_{h} and g_{KL} not only in octopus cells of multiple strains of mice but also between cell types (Cao and Oertel, 2011). Second, I_{h} and I_{KL} are balanced at the resting potential not only in the wild type but also in mutant mice. When I_{h} is reduced in HCN1 null mice, I_{KL} is correspondingly reduced so that I_{h} and I_{KL} remain roughly balanced (Cao and Oertel, 2011). The present experiments indicate that I_{h} and I_{KL} are balanced even when the ion channels are not uniform as revealed by differing pharmacological sensitivity of I_{KL} . Our finding that the balance of I_{h} and I_{KL} is precise at rest over an almost ten-fold range of g_{KL} and a four-fold range of g_{h} , when the subunit composition of the mediating ion channels varies, and when the genetic background varies suggests that the coregulation of g_{h} and g_{KL} may be through their activation at rest.

The present findings support our earlier conclusion that g_{h} somehow regulates g_{KL} . The conclusion was initially based on the finding that a reduction in g_{h} by the removal of one of the subunits of the channels led to a concomitant reduction in g_{KL} (Cao and Oertel, 2011). Removal of a prominent subunit of the channels that mediate g_{KL} is a test of that conclusion. *A priori*, one might predict that the removal of Kv1.1 might lead to a decrease in g_{KL} just as removal of HCN1 resulted in a decrease in g_{h} but if g_{h} governs g_{KL} , g_{KL} would not be expected to decrease. The present results clearly support the latter scenario. The loss of Kv1.1 was compensated. Neurons seem to differ in the mechanisms of coregulation and g_{h} does not always regulate g_{K} . In lobster stomatogastric neurons, for example, altering g_{K} resulted in a change of g_{h} ; overexpression of K^{+} channels of the Kv4 family led to an enhancement of I_{h} (MacLean et al., 2005; MacLean et al., 2003). We do not know whether

this difference reflects a difference between mammals and invertebrates. The extraordinary characteristics of mammalian octopus cells, whose unusually large resting conductances give them temporal precision in signaling, provide us with a rare opportunity to study the regulation of conductances that are too small to be measured at the resting potential in most mammalian neurons.

Acknowledgments

We are particularly grateful to Bruce Tempel who generously supplied us with the Kv1.1 mutant mice. We are also grateful for the colleagues who have made this work possible, especially Rebecca Welch and her effective office staff and Ravi Kochhar who kept our computers humming. We also thank Lin Lin for interesting discussion and for her thoughtful comments on multiple version of the manuscript. Steven Wiesner generously commented on one of the early versions of the manuscript. This work was supported by a grant from NIH DC00176 and by the Fall Research Competition from the University of Wisconsin.

Abbreviations

DNQX	6,7-dinitroquinoxaline-2,3-dione
DTX	dendrotoxin g_h hyperpolarization-activated, mixed cation conductance
g_{KL}	low-voltage-activated potassium conductance
I_h	hyperpolarization-activated, mixed cation current
I_{KL}	low-voltage-activated potassium current
TEA	tetraethylammonium
TTX	tetrodotoxin
VCN	ventral cochlear nucleus

References

- Adams PR, Brown DA. Synaptic inhibition of the M-current: slow excitatory post-synaptic potential mechanism in bullfrog sympathetic neurones. *J Physiol.* 1982; 332:263–272. [PubMed: 6984074]
- Altomare C, Terragni B, Brioschi C, Milanese R, Pagliuca C, Viscomi C, Moroni A, Baruscotti M, DiFrancesco D. Heteromeric HCN1–HCN4 channels: a comparison with native pacemaker channels from the rabbit sinoatrial node. *J Physiol.* 2003; 549:347–359. [PubMed: 12702747]
- Amarillo Y, Zagha E, Mato G, Rudy B, Nadal MS. The interplay of seven subthreshold conductances controls the resting membrane potential and the oscillatory behavior of thalamocortical neurons. *J Neurophysiol.* 2014; 112:393–410. [PubMed: 24760784]
- Amendola J, Woodhouse A, Martin-Eauclaire MF, Goaillard JM. Ca(2+)-cAMP-sensitive covariation of I(A) and I(H) voltage dependences tunes rebound firing in dopaminergic neurons. *J Neurosci.* 2012; 32:2166–2181. [PubMed: 22323729]
- Bagchi B, Al-Sabi A, Kaza S, Scholz D, O’Leary VB, Dolly JO, Ovsepian SV. Disruption of myelin leads to ectopic expression of K(V)1.1 channels with abnormal conductivity of optic nerve axons in a cuprizone-induced model of demyelination. *PLoS One.* 2014; 9:e87736. [PubMed: 24498366]
- Bal R, Oertel D. Hyperpolarization-activated, mixed-cation current (I_h) in octopus cells of the mammalian cochlear nucleus. *J Neurophysiol.* 2000; 84:806–817. [PubMed: 10938307]
- Bal R, Oertel D. Voltage-activated calcium currents in octopus cells of the mouse cochlear nucleus. *J Assoc Res Otolaryngol.* 2007; 8:509–521. [PubMed: 17710492]

- Bal R, Oertel D. Potassium currents in octopus cells of the mammalian cochlear nuclei. *J Neurophysiol.* 2001; 86:2299–2311. [PubMed: 11698520]
- Banks MI, Pearce RA, Smith PH. Hyperpolarization-activated cation current (I_h) in neurons of the medial nucleus of the trapezoid body: voltage-clamp analysis and enhancement by norepinephrine and cAMP suggest a modulatory mechanism in the auditory brain stem. *J Neurophysiol.* 1993; 70:1420–1432. [PubMed: 7506755]
- Barnes-Davies M, Barker MC, Osmani F, Forsythe ID. Kv1 currents mediate a gradient of principal neuron excitability across the tonotopic axis in the rat lateral superior olive. *Eur J Neurosci.* 2004; 19:325–333. [PubMed: 14725627]
- Begum R, Bakiri Y, Volynski KE, Kullmann DM. Action potential broadening in a presynaptic channelopathy. *Nat Commun.* 2016; 7:12102. [PubMed: 27381274]
- Berger C, Meyer EM, Ammer JJ, Felmy F. Large somatic synapses on neurons in the ventral lateral lemniscus work in pairs. *J Neurosci.* 2014; 34:3237–3246. [PubMed: 24573282]
- Bergquist S, Dickman DK, Davis GW. A hierarchy of cell intrinsic and target-derived homeostatic signaling. *Neuron.* 2010; 66:220–234. [PubMed: 20434999]
- Brew HM, Forsythe ID. Two voltage-dependent K⁺ conductances with complementary functions in postsynaptic integration at a central auditory synapse. *J Neurosci.* 1995; 15:8011–8022. [PubMed: 8613738]
- Brew HM, Forsythe ID. Systematic variation of potassium current amplitudes across the tonotopic axis of the rat medial nucleus of the trapezoid body. *Hear Res.* 2005; 206:116–132. [PubMed: 16081003]
- Brew HM, Gittelman JX, Silverstein RS, Hanks TD, Demas VP, Robinson LC, Robbins CA, Kee-Johnson J, Chiu SY, Messing A, Tempel BL. Seizures and reduced life span in mice lacking the potassium channel subunit Kv1.2, but hypoexcitability and enlarged Kv1 currents in auditory neurons. *J Neurophysiol.* 2007; 98:1501–1525. [PubMed: 17634333]
- Brew HM, Hallows JL, Tempel BL. Hyperexcitability and reduced low threshold potassium currents in auditory neurons of mice lacking the channel subunit Kv1.1. *J Physiol.* 2003; 548:1–20. [PubMed: 12611922]
- Cao XJ, Oertel D. Auditory nerve fibers excite targets through synapses that vary in convergence, strength, and short-term plasticity. *J Neurophysiol.* 2010; 104:2308–2320. [PubMed: 20739600]
- Cao XJ, Oertel D. The magnitudes of hyperpolarization-activated and low-voltage-activated potassium currents co-vary in neurons of the ventral cochlear nucleus. *J Neurophysiol.* 2011; 106:630–640. [PubMed: 21562186]
- Cao XJ, Shatadal S, Oertel D. Voltage-sensitive conductances of bushy cells of the mammalian ventral cochlear nucleus. *J Neurophysiol.* 2007; 97:3961–3975. [PubMed: 17428908]
- Cuttle MF, Rusznak Z, Wong AY, Owens S, Forsythe ID. Modulation of a presynaptic hyperpolarization-activated cationic current (I_h) at an excitatory synaptic terminal in the rat auditory brainstem. *J Physiol.* 2001; 534:733–744. [PubMed: 11483704]
- Dodson PD, Barker MC, Forsythe ID. Two heteromeric Kv1 potassium channels differentially regulate action potential firing. *J Neurosci.* 2002; 22:6953–6961. [PubMed: 12177193]
- Ferragamo MJ, Oertel D. Octopus cells of the mammalian ventral cochlear nucleus sense the rate of depolarization. *J Neurophysiol.* 2002; 87:2262–2270. [PubMed: 11976365]
- Fonseca RC, Hallows JL, Trimmer JS, Rhodes KJ, Tempel BL. Localization of Kv channels in the auditory system. *AssResOtolaryngolAbstr.* 1998; 213
- Gardner SM, Trussell LO, Oertel D. Time course and permeation of synaptic AMPA receptors in cochlear nuclear neurons correlate with input. *J Neurosci.* 1999; 19:8721–8729. [PubMed: 10516291]
- Gardner SM, Trussell LO, Oertel D. Correlation of AMPA receptor subunit composition with synaptic input in the mammalian cochlear nuclei. *J Neurosci.* 2001; 21:7428–7437. [PubMed: 11549753]
- Gittelman JX, Tempel BL. Kv1.1-containing channels are critical for temporal precision during spike initiation. *J Neurophysiol.* 2006; 96:1203–1214. [PubMed: 16672305]
- Golding NL, Oertel D. Synaptic integration in dendrites: exceptional need for speed. *J Physiol.* 2012; 590:5563–5569. [PubMed: 22930273]

- Golding NL, Robertson D, Oertel D. Recordings from slices indicate that octopus cells of the cochlear nucleus detect coincident firing of auditory nerve fibers with temporal precision. *J Neurosci.* 1995; 15:3138–3153. [PubMed: 7722652]
- Golowasch J. Ionic Current Variability and Functional Stability in the Nervous System. *Bioscience.* 2014; 64:570–580. [PubMed: 26069342]
- Golowasch J, Abbott LF, Marder E. Activity-dependent regulation of potassium currents in an identified neuron of the stomatogastric ganglion of the crab *Cancer borealis*. *J Neurosci.* 1999a; 19:RC33. [PubMed: 10516335]
- Golowasch J, Casey M, Abbott LF, Marder E. Network stability from activity-dependent regulation of neuronal conductances. *Neural Comput.* 1999b; 11:1079–1096. [PubMed: 10418158]
- Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD, Chandy KG. Pharmacological characterization of five cloned voltage-gated K⁺ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. *Mol Pharmacol.* 1994; 45:1227–1234. [PubMed: 7517498]
- Grunnet M, Rasmussen HB, Hay-Schmidt A, Rosenstjerne M, Klaerke DA, Olesen SP, Jespersen T. KCNE4 is an inhibitory subunit to Kv1.1 and Kv1.3 potassium channels. *Biophys J.* 2003; 85:1525–1537. [PubMed: 12944270]
- Halliwel JV, Adams PR. Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res.* 1982; 250:71–92. [PubMed: 6128061]
- Harris NC, Constanti A. Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons in vitro. *J Neurophysiol.* 1995; 74:2366–2378. [PubMed: 8747199]
- Harvey AL. Recent studies on dendrotoxins and potassium ion channels. *Gen Pharmacol.* 1997; 28:7–12. [PubMed: 9112070]
- Harvey AL. Twenty years of dendrotoxins. *Toxicon.* 2001; 39:15–26. [PubMed: 10936620]
- Hopkins WF. Toxin and subunit specificity of blocking affinity of three peptide toxins for heteromultimeric, voltage-gated potassium channels expressed in *Xenopus* oocytes. *J Pharmacol & Exp Therapeut.* 1998; 285:1051–1060.
- Hopkins WF, Allen ML, Houamed KM, Tempel BL. Properties of voltage-gated K⁺ currents expressed in *Xenopus* oocytes by mKv1.1, mKv1.2 and their heteromultimers as revealed by mutagenesis of the dendrotoxin-binding site in mKv1.1. *Pflugers. Archiv. - Eur J Physiol.* 1994; 428:382–390.
- Huang Z, Lujan R, Kadurin I, Uebele VN, Renger JJ, Dolphin AC, Shah MM. Presynaptic HCN1 channels regulate Cav3.2 activity and neurotransmission at select cortical synapses. *Nat Neurosci.* 2011; 14:478–486. [PubMed: 21358644]
- Huang Z, Walker MC, Shah MM. Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. *J Neurosci.* 2009; 29:10979–10988. [PubMed: 19726656]
- Hyun JH, Eom K, Lee KH, Ho WK, Lee SH. Activity-dependent downregulation of D-type K⁺ channel subunit Kv1.2 in rat hippocampal CA3 pyramidal neurons. *J Physiol.* 2013; 591:5525–5540. [PubMed: 23981714]
- Jenkins PM, McIntyre JC, Zhang L, Anantharam A, Vesely ED, Arendt KL, Carruthers CJ, Kerppola TK, Iniguez-Lluhi JA, Holz RW, Sutton MA, Martens JR. Subunit-dependent axonal trafficking of distinct alpha heteromeric potassium channel complexes. *J Neurosci.* 2011; 31:13224–13235. [PubMed: 21917805]
- Karcz A, Hennig MH, Robbins CA, Tempel BL, Rubsamen R, Kopp-Scheinpflug C. Low-voltage activated Kv1.1 subunits are crucial for the processing of sound source location in the lateral superior olive in mice. *J Physiol.* 2011; 589:1143–1157. [PubMed: 21224222]
- Khakh BS, Henderson G. Hyperpolarization-activated cationic currents (I_h) in neurones of the trigeminal mesencephalic nucleus of the rat. *J Physiol (Lond).* 1998; 510:695–704. [PubMed: 9660886]
- Khorkova O, Golowasch J. Neuromodulators, not activity, control coordinated expression of ionic currents. *J Neurosci.* 2007; 27:8709–8718. [PubMed: 17687048]
- Khurana S, Remme MW, Rinzel J, Golding NL. Dynamic interaction of I_h and I_K-LVA during trains of synaptic potentials in principal neurons of the medial superior olive. *J Neurosci.* 2011; 31:8936–8947. [PubMed: 21677177]

- Kim YH, Holt JR. Functional contributions of HCN channels in the primary auditory neurons of the mouse inner ear. *J Gen Physiol.* 2013; 142:207–223. [PubMed: 23980193]
- Koch U, Braun M, Kapfer C, Grothe B. Distribution of HCN1 and HCN2 in rat auditory brainstem nuclei. *Eur J Neurosci.* 2004; 20:79–91. [PubMed: 15245481]
- Kuba H, Yamada R, Fukui I, Ohmori H. Tonotopic specialization of auditory coincidence detection in nucleus laminaris of the chick. *J Neurosci.* 2005; 25:1924–1934. [PubMed: 15728832]
- Liu Q, Manis PB, Davis RL. Ih and HCN channels in murine spiral ganglion neurons: tonotopic variation, local heterogeneity, and kinetic model. *J Assoc Res Otolaryngol.* 2014; 15:585–599. [PubMed: 24558054]
- Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. A family of hyperpolarization-activated mammalian cation channels. *Nature.* 1998; 393:587–591. [PubMed: 9634236]
- Lujan R, de Cabo de la Vega, Dominguez del TE, Ballesta JJ, Criado M, Juiz JM. Immunohistochemical localization of the voltage-gated potassium channel subunit Kv1.4 in the central nervous system of the adult rat. *J Chem Neuroanat.* 2003; 26:209–224. [PubMed: 14615029]
- Luthi A, McCormick DA. Periodicity of thalamic synchronized oscillations: the role of Ca²⁺-mediated upregulation of Ih. *Neuron.* 1998; 20:553–563. [PubMed: 9539128]
- Maccaferri G, McBain CJ. The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurons. *J Physiol (Lond).* 1996; 497:119–130. [PubMed: 8951716]
- MacLean JN, Zhang Y, Goeritz ML, Casey R, Oliva R, Guckenheimer J, Harris-Warrick RM. Activity-independent coregulation of IA and Ih in rhythmically active neurons. *J Neurophysiol.* 2005; 94:3601–3617. [PubMed: 16049145]
- MacLean JN, Zhang Y, Johnson BR, Harris-Warrick RM. Activity-independent homeostasis in rhythmically active neurons. *Neuron.* 2003; 37:109–120. [PubMed: 12526777]
- Manganas LN, Trimmer JS. Subunit composition determines Kv1 potassium channel surface expression. *J Biol Chem.* 2000; 275:29685–29693. [PubMed: 10896669]
- Manis PB, Marx SO. Outward currents in isolated ventral cochlear nucleus neurons. *J Neurosci.* 1991; 11:2865–2880. [PubMed: 1880553]
- Mathews PJ, Jercog PE, Rinzel J, Scott LL, Golding NL. Control of submillisecond synaptic timing in binaural coincidence detectors by K(v)1 channels. *Nat Neurosci.* 2010; 13:601–609. [PubMed: 20364143]
- McGinley MJ, Liberman MC, Bal R, Oertel D. Generating synchrony from the asynchronous: compensation for cochlear traveling wave delays by the dendrites of individual brainstem neurons. *J Neurosci.* 2012; 32:9301–9311. [PubMed: 22764237]
- McGinley MJ, Oertel D. Rate thresholds determine the precision of temporal integration in principal cells of the ventral cochlear nucleus. *Hear Res.* 2006; 216–217:52–63.
- Mo ZL, Adamson CL, Davis RL. Dendrotoxin-sensitive K(+) currents contribute to accommodation in murine spiral ganglion neurons. *J Physiol.* 2002; 542:763–778. [PubMed: 12154177]
- Mo ZL, Davis RL. Heterogeneous voltage dependence of inward rectifier currents in spiral ganglion neurons. *J Neurophysiol.* 1997; 78:3019–3027. [PubMed: 9405521]
- Moosmang S, Stieber J, Zong X, Biel M, Hofmann F, Ludwig A. Cellular expression and functional characterization of four hyperpolarization-activated pacemaker channels in cardiac and neuronal tissues. *Eur J Biochem.* 2001; 268:1646–1652. [PubMed: 11248683]
- Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, Yin D, Thompson RF, Siegelbaum SA, Kandel ER, Morozov A. The hyperpolarization-activated HCN1 channel is important for motor learning and neuronal integration by cerebellar Purkinje cells. *Cell.* 2003; 115:551–564. [PubMed: 14651847]
- O’Leary T, van Rossum MC, Wyllie DJ. Homeostasis of intrinsic excitability in hippocampal neurones: dynamics and mechanism of the response to chronic depolarization. *J Physiol.* 2010; 588:157–170. [PubMed: 19917565]
- O’Leary T, Williams AH, Caplan JS, Marder E. Correlations in ion channel expression emerge from homeostatic tuning rules. *Proc Natl Acad Sci USA.* 2013; 110:E2645–E2654. [PubMed: 23798391]

- O'Leary T, Williams AH, Franci A, Marder E. Cell types, network homeostasis, and pathological compensation from a biologically plausible ion channel expression model. *Neuron*. 2014; 82:809–821. [PubMed: 24853940]
- Oertel D. Synaptic responses and electrical properties of cells in brain slices of the mouse anteroventral cochlear nucleus. *J Neurosci*. 1983; 3:2043–2053. [PubMed: 6619923]
- Oertel D, Bal R, Gardner SM, Smith PH, Joris PX. Detection of synchrony in the activity of auditory nerve fibers by octopus cells of the mammalian cochlear nucleus. *Proc Nat Acad Sci USA*. 2000; 97:11773–11779. [PubMed: 11050208]
- Oertel D, Shatadal S, Cao XJ. In the ventral cochlear nucleus Kv1.1 and subunits of HCN1 are colocalized at surfaces of neurons that have low-voltage-activated and hyperpolarization-activated conductances. *Neurosci*. 2008; 154:77–86.
- Ovsepian SV, LeBerre M, Steuber V, O'Leary VB, Leibold C, Dolly JO. Distinctive role of Kv1.1 subunit in the biology and functions of low threshold K⁺ channels with implications for neurological disease. *Pharmacol Ther*. 2016; 159:93–101. [PubMed: 26825872]
- Owen DG, Hall A, Stephens G, Stow J, Robertson B. The relative potencies of dendrotoxins as blockers of the cloned voltage-gated K⁺ channel, mKv1.1 (MK-1), when stably expressed in Chinese hamster ovary cells. *Br J Pharmacol*. 1997; 120:1029–1034. [PubMed: 9134213]
- Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Ann Rev Physiol*. 1996; 58:299–327. 299–327. [PubMed: 8815797]
- Rasband MN, Trimmer JS. Subunit composition and novel localization of K⁺ channels in spinal cord. *J Comp Neurol*. 2001; 429:166–176. [PubMed: 11086297]
- Rathouz M, Trussell L. Characterization of outward currents in neurons of the avian nucleus magnocellularis. *J Neurophysiol*. 1998; 80:2824–2835. [PubMed: 9862887]
- Robbins CA, Tempel BL. Kv1.1 and Kv1.2: similar channels, different seizure models. *Epilepsia*. 2012; 53(Suppl 1):134–141.
- Robertson B, Owen D, Stow J, Butler C, Newland C. Novel effects of dendrotoxin homologues on subtypes of mammalian Kv1 potassium channels expressed in *Xenopus* oocytes. *FEBS Lett*. 1996; 383:26–30. [PubMed: 8612784]
- Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: From molecules to physiological function. *Ann Rev Physiol*. 2003; 65:453–480. [PubMed: 12471170]
- Rothman JS, Manis PB. Kinetic analyses of three distinct potassium conductances in ventral cochlear nucleus neurons. *J Neurophysiol*. 2003a; 89:3083–3096. [PubMed: 12783952]
- Rothman JS, Manis PB. The roles potassium currents play in regulating the electrical activity of ventral cochlear nucleus neurons. *J Neurophysiol*. 2003b; 89:3097–3113. [PubMed: 12783953]
- Rusznak Z, Bakondi G, Pocsai K, Por A, Kosztka L, Pal B, Nagy D, Szucs G. Voltage-gated potassium channel (Kv) subunits expressed in the rat cochlear nucleus. *J Histochem Cytochem*. 2008; 56:443–465. [PubMed: 18256021]
- Rusznak Z, Forsythe ID, Stanfield PR. Characterization of the hyperpolarization activated nonspecific cation current (I_h) of bushy neurones from the rat anteroventral cochlear nucleus studied in a thin brain slice preparation. *Neurobiology (Budapest)*. 1996; 4:275–276.
- Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, Tibbs GR. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell*. 1998; 93:717–729. [PubMed: 9630217]
- Scott LL, Mathews PJ, Golding NL. Posthearing developmental refinement of temporal processing in principal neurons of the medial superior olive. *J Neurosci*. 2005; 25:7887–7895. [PubMed: 16135745]
- Shepherd JD, Rumbaugh G, Wu J, Chowdhury S, Plath N, Kuhl D, Huganir RL, Worley PF. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. *Neuron*. 2006; 52:475–484. [PubMed: 17088213]
- Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron*. 1998; 20:809–819. [PubMed: 9581771]

- Soh H, Pant R, LoTurco JJ, Tzingounis AV. Conditional deletions of epilepsy-associated KCNQ2 and KCNQ3 channels from cerebral cortex cause differential effects on neuronal excitability. *J Neurosci.* 2014; 34:5311–5321. [PubMed: 24719109]
- Stuhmer W, Ruppersberg JP, Schroter KH, Sakmann B, Stocker M, Giese KP, Perschke A, Baumann A, Pongs O. Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *EMBO J.* 1989; 8:3235–3244. [PubMed: 2555158]
- Svirskis G, Kotak V, Sanes DH, Rinzel J. Sodium along with low-threshold potassium currents enhance coincidence detection of subthreshold noisy signals in MSO neurons. *J Neurophysiol.* 2004; 91:2465–2473. [PubMed: 14749317]
- Swensen AM, Bean BP. Robustness of burst firing in dissociated purkinje neurons with acute or long-term reductions in sodium conductance. *J Neurosci.* 2005; 25:3509–3520. [PubMed: 15814781]
- Thayer DA, Yang SB, Jan YN, Jan LY. N-linked glycosylation of Kv1.2 voltage-gated potassium channel facilitates cell surface expression and enhances the stability of internalized channels. *J Physiol.* 2016
- Thiagarajan TC, Lindskog M, Tsien RW. Adaptation to synaptic inactivity in hippocampal neurons. *Neuron.* 2005; 47:725–737. [PubMed: 16129401]
- Thiagarajan TC, Piedras-Renteria ES, Tsien RW. alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron.* 2002; 36:1103–1114. [PubMed: 12495625]
- Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* 1999; 22:221–227. [PubMed: 10322495]
- Turrigiano GG. Homeostatic signaling: the positive side of negative feedback. *Curr Opin Neurobiol.* 2007; 17:1–7.
- Ulen C, Tytgat J. Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem.* 2001; 276:6069–6072. [PubMed: 11133998]
- Vahasoyrinki M, Niven JE, Hardie RC, Weckstrom M, Juusola M. Robustness of neural coding in *Drosophila* photoreceptors in the absence of slow delayed rectifier K⁺ channels. *J Neurosci.* 2006; 26:2652–2660. [PubMed: 16525044]
- Wang FC, Bell N, Reid P, Smith LA, McIntosh P, Robertson B, Dolly JO. Identification of residues in dendrotoxin K responsible for its discrimination between neuronal K⁺ channels containing Kv1.1 and 1.2 alpha subunits. *Eur J Biochem.* 1999a; 263:222–229. [PubMed: 10429207]
- Wang FC, Parcej DN, Dolly JO. alpha subunit compositions of Kv1.1-containing K⁺ channel subtypes fractionated from rat brain using dendrotoxins. *Eur J Biochem.* 1999b; 263:230–237. [PubMed: 10429208]
- Watanabe I, Zhu J, Recio-Pinto E, Thornhill WB. Glycosylation affects the protein stability and cell surface expression of Kv1.4 but Not Kv1.1 potassium channels. A pore region determinant dictates the effect of glycosylation on trafficking. *J Biol Chem.* 2004; 279:8879–8885. [PubMed: 14688283]
- Watanabe I, Zhu J, Recio-Pinto E, Thornhill WB. The Degree of N-glycosylation Affects the Trafficking and Cell Surface Expression Levels of Kv1.4 Potassium Channels. *J Membr Biol.* 2015; 248:187–196. [PubMed: 25416425]
- Watanabe I, Zhu J, Sutachan JJ, Gottschalk A, Recio-Pinto E, Thornhill WB. The glycosylation state of Kv1.2 potassium channels affects trafficking, gating, and simulated action potentials. *Brain Res.* 2007; 1144:1–18. [PubMed: 17324383]
- Werkman TR, Gustafson TA, Rogowski RS, Blaustein MP, Rogawski MA. Tityustoxin-K alpha, a structurally novel and highly potent K⁺ channel peptide toxin, interacts with the alpha-dendrotoxin binding site on the cloned Kv1.2 K⁺ channel. *Mol Pharmacol.* 1993; 44:430–436. [PubMed: 8355670]
- Wheeler DG, Barrett CF, Groth RD, Safa P, Tsien RW. CaMKII locally encodes L-type channel activity to signal to nuclear CREB in excitation-transcription coupling. *J Cell Biol.* 2008; 183:849–863. [PubMed: 19047462]
- Whitaker GM, Angoli D, Nazzari H, Shigemoto R, Accili EA. HCN2 and HCN4 isoforms self-assemble and co-assemble with equal preference to form functional pacemaker channels. *J Biol Chem.* 2007; 282:22900–22909. [PubMed: 17553794]

- Wu SH. Physiological properties of neurons in the ventral nucleus of the lateral lemniscus of the rat: Intrinsic membrane properties and synaptic responses. *J Neurophysiol.* 1999; 81:2862–2874. [PubMed: 10368403]
- Zhao S, Golowasch J. Ionic current correlations underlie the global tuning of large numbers of neuronal activity attributes. *J Neurosci.* 2012; 32:13380–13388. [PubMed: 23015428]
- Zhou L, Zhang CL, Messing A, Chiu SY. Temperature-sensitive neuromuscular transmission in Kv1.1 null mice: role of potassium channels under the myelin sheath in young nerves. *J Neurosci.* 1998; 18:7200–7215. [PubMed: 9736643]

Highlights

- The large size of g_h and g_{KL} in octopus cells allowed us to resolve and measure I_h and I_{KL} at the resting potential.
- Perturbing the magnitude and subunit composition of these conductances genetically did not affect average resting potentials.
- I_{KL} balanced I_h at rest in all octopus cells, regardless of mouse strain and individual resting potential.
- Instead of g_h and g_{KL} regulating the resting potential, it seems that the resting potential regulates g_h and g_{KL} .

Figure 1.

Functional roles of g_{KL} and g_h are demonstrated by blocking those conductances. **A:** (Left panel) Whole-cell patch-clamp responses from the cell body of an octopus cell to depolarizing current pulses increased in 1 nA steps illustrate the strong rectification and small action potentials that are characteristic of octopus cells. Response to 3.5 nA hyperpolarizing current pulse sags back toward rest. (Middle panel) Blocking g_{KL} with the application of 50 nM α -DTX depolarized the cell by 6 mV, increased its input resistance and caused it to fire repetitively (green trace). The larger hyperpolarization to the 3.5 nA hyperpolarizing current pulse reflects the cell's increased input resistance. (Right panel) The increase in size and duration of the first action potential and the shapes of subsequent action potentials are shown on an expanded time scale in the absence (black) and presence (green) of α -DTX. **B:** (Left panel) Responses of a different octopus cell to hyperpolarizing current pulses increased in 0.5 nA steps show how strong hyperpolarizations first hyperpolarize the cell and that then the membrane voltage sags back toward rest. The cell fired action potentials after the offset of hyperpolarizing currents. (Middle panel) Blocking g_h with 50 μ M ZD7288 removed the sag in responses to hyperpolarization. The size of the response evoked by a 3 nA depolarizing current was larger than before reflecting an increase in input resistance. (Right panel) Superimposition of responses to hyperpolarizing current pulses under control conditions and in the presence of 50 μ M ZD7288 show that the cell's voltage changes were larger and slower in the presence of ZD7288.

Figure 2.

Resting membrane potentials of octopus cells do not differ significantly between strains of mice. Mean resting potentials lay within a 3 mV range. These measurements of were made in current-clamp with patch pipettes that contained 108 mM K⁺ gluconate when slices were bathed in saline that contained 4.2 mM K⁺ in the absence of blockers.

Figure 3.

I_{KL} and I_h in octopus cells. **A:** In an octopus cell from a *Kv1.1* null mutant and from a wild type control, voltage pulses from -90 mV to -40 mV evoked outward currents of roughly equal steady-state magnitudes but the peak current was larger in the wild type cell. **B:** Similar depolarizing voltage pulses in an *HCN1* null mutant evoked a smaller current than in the wild type but the shape of the current was similar. **C:** Peak outward currents, but not steady state outward currents, were significantly larger in the wild type than in *Kv1.1* null octopus cells. **D:** The voltage activation ranges were similar in *Kv1.1* null mutant octopus cells and their wild type controls as in *HCN1* null mutants and their wild type controls. In all peak currents were activated at around -70 mV, indicating that in mutants as in the wild type, the outward current is low-voltage-activated, that it is I_{KL} . **E:** In an octopus cell from a *Kv1.1* null mutant mouse and a wild type control, voltage steps from -57 mV to -127 mV evoked first a step and then a gradual increase in I_h . The magnitude and shape of I_h was similar in the two cells. **F:** In an *HCN1* null mutant mouse, hyperpolarization-activated currents activated by steps from -57 mV to -127 mV were smaller and slower than in the wild type. **G:** Averaged current-voltage relationship of octopus cells from 3 wild-type and 3

Kv1.1 null mutants reveal no significant differences. **H:** The magnitudes of currents activated by depolarization from -90 mV to -40 mV, an estimate of I_{KL} , and those activated by hyperpolarization from -57 mV to -122 mV, an estimate of I_h , co-varied between strains of mice ($R = 0.95$). The measurements in ICR mice, HCN1 null mutants and their wild type controls were reported in a previous publication, Figure 7 in (Cao and Oertel, 2011). All recordings were made in the presence of 1 μ M TTX, 0.25 mM CdCl_2 , 40 μ M DNQX, and 1 μ M strychnine; 50 μ M ZD7288 was added to measure I_{KL} and 50 nM α -DTX was added to measure I_h . In this figure and all subsequent figures, traces from Kv1.1 null mutants are shown in red, traces from HCN1 null mutants are shown in blue, and their wild type controls are shown in black.

Figure 4.

I_{KL} in octopus cells of Kv1.1 null mutants and their wild type controls differs in sensitivity to pharmacological blockers. **A:** In a wild type octopus cell, depolarizing voltage steps evoked outward currents. Application of 50 nM α -DTX partially blocked those outward currents. The current-voltage relationship of peak currents from several cells ($n=4$), shows that under control conditions, depolarization to more than -70 mV evoked outward currents but that in the presence of α -DTX, peak current responses were linear to about -55 mV (dashed line). α -DTX thus blocked most I_{KL} , leaving a high-voltage-activated outward current. **B:** Similar measurements from an octopus cell from a Kv1.1 null mutant mouse show that the total outward current was smaller than that in a wild type control cell. In the presence of α -DTX the averaged current-voltage relationship ($n=5$) remains non-linear in the voltage range between -70 and -50 mV showing that α -DTX does not block I_{KL} in Kv1.1 null mutant octopus cells.

Figure 5.

I_h at the resting potential is larger than the α -DTX sensitive portion of I_{KL} in all octopus cells tested. Each panel illustrates an experiment in a single octopus cell; panels illustrate experiments from octopus cells from different mouse strains. Each cell was held in voltage-clamp at its resting potential, given at the upper left of each panel, so that under control conditions no holding current was required. When 50 μ M ZD7288 was applied, an unbalanced outward holding current was observed whose magnitude reflects the magnitude of the blocked I_h . In the continued presence of ZD7288, the application of 50 nM α -DTX reduced the outward holding current but did not bring it to 0 nA. The proportion of the unbalanced, residual current was greater in Kv1.1 null mutants than in the other strains.

Figure 6.

I_h , which is sensitive ZD7288, is balanced by the sum of two components of I_{KL} , one being sensitive to 50 nM α -DTX, the other being insensitive to α -DTX but sensitive to 10 mM TEA. **A, B:** Blocking I_h with 50 μ M ZD7288 resulted in an outward holding current. That holding current was reduced by 50 nM α -DTX. 10 mM TEA blocked the remaining outward current. The proportion of the current that was insensitive to α -DTX was smaller in the ICR than in the Kv1.1 octopus cell.

Figure 7.

Comparison of the sensitivity of octopus cells to the rate at which they are depolarized across mouse strains. **A:** (Left) An octopus cell from a Kv1.1 wild type control mouse was depolarized with current pulses that rose to 1 nA with ramps of varying slope (C). The cell fired action potentials only when the rate of depolarization exceeded a threshold level that is indicated with the dark subthreshold and suprathreshold traces. (Right) Peak depolarization is plotted as a function of the slope of linear fits to the subthreshold rising phase of the voltage response to ramps of current (dV/dt). The threshold dV/dt is shown with a dashed line. **B:** A similar measurement in an octopus cell of a Kv1.1 null mouse revealed a lower rate threshold. **C:** Representation of a family of ramped current pulses that was used to depolarize octopus cells as in A and B. **D:** Averaged rate thresholds indicate that the presence of Kv1.1 and HCN1 significantly increases the rate thresholds. (* Denotes statistical difference $p < 0.05$; ** denotes statistical difference $p < 0.001$.) **E:** The dV/dt thresholds, measured in current clamp as shown above in various strains of mice vary as a function of the magnitude of I_{KL} estimated in separate experiments under voltage-clamp. The estimate of I_{KL} was obtained by measuring the peak outward current in response to voltage steps from -90 mV to -40 mV in the presence of $40 \mu\text{M}$ DNQX, $1 \mu\text{M}$ strychnine, $1 \mu\text{M}$ TTX, 0.25 mM CdCl₂, and $50 \mu\text{M}$ ZD7288. There was a positive correlation between the estimate of I_{KL} and dV/dt threshold, $R = 0.85$. Octopus cells of HCN1 wild type control

mice have the largest I_{KL} and also have the highest dV/dt threshold, whereas bushy cells of Kv1.1 null mice have a small I_{KL} and a low dV/dt threshold.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Comparison of biophysical properties of octopus cells in different mouse strains.

	Max g _{KL} (nS)	Max g _h (nS)	R _{input} (MΩ)	τ (msec)	dV/dt threshold (mV/msec)
ICR	419 ± 87 (4) 514 ± 135 (12) ¹	116 ± 38 (4) 150 ± 30 (36) ²	6.7 ± 2.0 (56) ²	0.21 ± 0.07 (20) ²	9.5 ± 3.6 (7)
HCN1 ^{+/+}	90 ± 28 (4)	61 ± 24 (5)	14 ± 2 (8) ³	0.3 ± 0.2 (13) ³	6.6 ± 0.4 (4)
HCN1 ^{-/-}	56 ± 19 (3)	32 ± 11 (4)	19 ± 3 (19) ³	0.5 ± 0.1 (5) ³	5.3 ± 0.3 (5)
Kv1.1 ^{+/+}	60 ± 20 (5)	53 ± 19 (5)	13.6 ± 3.7 (5)	1.1 ± 0.2 (5)	4 ± 1.6 (5)
Kv1.1 ^{-/-}	45 ± 13 (4)	49 ± 22 (5)	14.8 ± 7.4 (4)	1.3 ± 0.3 (4)	2.4 ± 0.1 (5)

Table 1 legend. Values are given as means ± standard deviation. Numbers of cells from which measurements are derived are given in parentheses. To enable comparisons we have included measurements on ICR mice and HCN1 null mutants and their wild type controls from earlier publications. Those are indicated with superscripts:

¹ (Bal and Oertel, 2001);
² (Bal and Oertel, 2000);
³ (Cao and Oertel, 2011).

Table 2

Quantification of currents at the resting potential measured as illustrated in Figure 5.

	<i>a)</i> With ZD7288 (nA)	<i>b)</i> With ZD7288 and α -DTX (nA)	<i>b/a</i>	# Cells
ICR	1.09 ± 0.16	0.12 ± 0.04	11%	5
HCN1 ^{+/+}	0.6 ± 0.19	0.08 ± 0.01	13%	5
HCN1 ^{-/-}	0.34 ± 0.11	0.06 ± 0.01	18%	5
Kv1.1 ^{+/+}	0.32 ± 0.05	0.03 ± 0.02	10%	5
Kv1.1 ^{-/-}	0.26 ± 0.04	0.14 ± 0.02	54%	4

Table 2 legend. Resting currents in octopus cells from five strains of mice.

a) The magnitude of the outward current after 50 μ M ZD7288 was applied to an octopus cell whose voltage was held at its resting potential.

b) The magnitude of the outward current that remained after the application of 50 nM α -DTX.

b/a the ratio of the α -DTX-insensitive current/ZD7288-sensitive current.

Cells: Number of cells from which measurements were made.