Formation of complexes between ion channels is important for signal processing in the brain. Here we investigate the biochemical and biophysical interactions between HCN1 channels and Cav3.2 T-type channels. We found that HCN1 co-immunoprecipitated with Cav3.2 from lysates of either mouse brain or tsA-201 cells, with the HCN1 N-terminus associating with the Cav3.2 N-terminus. Cav3.2 channel activity appeared to be functionally regulated by HCN1. The expression of HCN1 induced a decrease in Cav3.2 Ba$^{2+}$ influx (I$_{Ba}$) along with altered channel kinetics and a depolarizing shift in activation gating. However, a reciprocal regulation of HCN1 by Cav3.2 was not observed. This study highlights a regulatory role of HCN1 on Cav3.2 voltage-dependent properties, which are expected to affect physiologic functions such as synaptic transmission and cellular excitability.
excitability. Previous studies from Huang et al., revealed a colocalization of HCN1 with Cav3.2, but not Cav3.1, at synapses of entorhinal cortical neurons. To examine whether this colocalization may be due to a physical association between Cav3 and HCN channels, we performed a series of co-IPs using mouse brain lysates. Co-IP experiments revealed that HCN1 channels immuno-precipitated with Cav3.2, but not Cav3.1 or Cav3.3 T-type channels (Fig. 1A), in agreement with previous co-localization work (4). In contrast, HCN2 channels were not found to be associated with any of T-type subunits (Fig. 1B). These data thus indicate a selective interaction between HCN1 and Cav3.2 channel subtypes.

**Co-expression of HCN1 alters Cav3.2 T-type channel activity**

It has been reported that the HCN channels regulate inactivation of T-type channels in hippocampal and entorhinal cortical neurons. We thus tested whether the observed biochemical interactions are reflected in a change in Cav3.2 channel function. Whole-cell voltage clamp recordings were performed from tsA-201 cells transiently transfected with Cav3.2 cDNA in the presence or absence of HCN1 cDNA constructs. The current density-voltage relationship for Cav3.2 activation was then examined using whole cell patch clamp (Fig. 2A, B), using ionic conditions in which HCN channels are functionally blocked (see Materials and methods). In the presence of HCN1, cells exhibited a decrease in Cav3.2 peak barium current across a wide range of voltages (between −45 to 30 mV; peak current density at −20 mV step, without HCN1: −91.3 ± 14.2 pA/pF, with HCN1: −44.8 ± 7.3 pA/pF, Fig. 2B, p < 0.005). In addition, there was a reduction in maximal slope conductance in the presence of HCN1 (without HCN1: 1.7 ± 0.2 pS/pF, with HCN1: 0.9 ± 0.1 pS/pF, Fig. 2G, p < 0.005). The kinetics of Cav3.2 channel activation and inactivation were determined by fitting the onset and decay of the current with mono-exponential functions. The time constant of inactivation was significantly slower in HCN1 co-expressing cells at a test potential of −20 mV (without HCN1: 17.7 ± 0.4 ms, with HCN1: 27.8 ± 3.1 ms, Fig. 2D, p < 0.05). However, the activation kinetics were not affected (Fig. 2C, p > 0.05). In addition, HCN1 co-expression shifted the half-activation voltage of Cav3.2 to more depolarized potentials ($V_{0.5act}$ for the recordings without HCN1: −37.1 ± 1.7 mV, with HCN1: −32.1 ± 1.5 mV, Fig. 2E and F, p < 0.05). The coexpression of HCN1 did not affect the steady-state inactivation curves of Cav3.2 (Fig. 2H, p > 0.05). These results suggest that HCN1 functionally interacts with Cav3.2 channels by altering Cav3.2 current density and Cav3.2 gating characteristics. To ensure that the effects of HCN1 on peak current density were not due to an expression artifact, we performed a series of experiments in which we coexpressed HCN2 with Cav3.2. At a test potential of −10 mV, the peak current densities for Cav3.2 channels alone or in the presence of HCN2 were, respectively, 46.8 ± 5.4 pA/pF (n = 10) and 51.9 ± 7.3 pA/pF (n=10, p = 0.8), and there was no effect on Gmax (data not shown). These data indicate that HCN2 channels which are unable to associate with Cav3.2 are unable to significantly suppress Cav3.2 channel activity.
Examination of interaction sites between HCN1 and Cav3.2 channels

To examine the channel regions that are involved in the interaction between HCN1 and Cav3.2, we used GFP-tagged intracellular regions of Cav3.2 channels which were co-transfected with full length HCN1 channels in tsA-201 cells. In this preparation, GFP-tagged Cav3.2 I-II linker, II-III linker, III-IV linker, N-terminus and C-terminus were tested. A series of co-IPs were performed to examine which intracellular region of Cav3.2 channels was associated with full length HCN1 channels. As a positive control we transfected full length Cav3.2. Consistent with the data in Fig. 1, Cav3.2 channels interacted with HCN1 when expressed heterologously (Fig. 3A). In addition, a
strong interaction between the Cav3.2 N-terminus and HCN1 was observed (Fig. 3B). However, none of the other tested Cav3.2 domain linkers nor the C-terminus were able to associate with HCN1 (Fig. 3). To further refine the binding region in the Cav3.2 N-terminus, we took advantage of Cav3.2 N1-terminus (residues 1–50), N2-terminus (residues 51–100) and N3-terminus (residues 25–75) constructs that were described previously. We observed a strong band from the co-IP of HCN1 channels with the Cav3.2 N3-terminus compared with N1- or N2-termini (Fig. 3C). To map the binding region in HCN1 channels, a similar approach was used. The cells were co-transfected with either mKate-tagged HCN1-N terminus or mKate-tagged HCN1-C terminus, and full length of Cav3.2 channels. As shown in Fig. 3D, Cav3.2 interacted with HCN1 N-terminus (Fig. 3D). These results provide strong evidence that the interaction between HCN1 and Cav3.2 involve the N-terminal regions of these channels.

**There is no reciprocal regulation of HCN1 channel activity by Cav3.2**

It has been reported that Ca$^{2+}$ influx regulates HCN1 channel activity. We thus wished to explore
whether there might be a reciprocal regulation of HCN1 channel properties by Cav3.2 channels. To determine the impact of Ca\(^{2+}\) influx through Cav3.2 channels on HCN1 channel activity, Ca\(^{2+}\) was removed from the intracellular solution, and cells were bathed in an external recording solution containing 2.5 mM Ca\(^{2+}\) to allow for T-type channel-mediated Ca\(^{2+}\) entry. When recording from T-type channels, cells are usually held at \(-100\) mV to recover channels from voltage-dependent inactivation. However, this is also the voltage at which HCN1 channels are activated. Therefore, to minimize the leak \(I_h\) current, cells were held at \(-50\) mV instead of \(-100\) mV, followed by a hyperpolarizing pulse (P1) to \(-100\) mV to remove inactivation of Cav3.2 and to measure \(I_h\). Then a depolarizing step to \(-20\) mV was applied to maximally activate Cav3.2 channels followed by a second hyperpolarizing pulse (P2) to again assess \(I_h\) (Fig. 4A). As shown in Fig. 4A, the activation of Cav3.2 current did not affect HCN1 current amplitude (HCN1: P2/P1 = 0.95 ± 0.05, n = 9; HCN1 + Cav3.2: P2/P1 = 0.93 ± 0.04, n = 11). Furthermore, there was also no effect of Cav3.2 coexpression on \(I_h\) current density (Fig. 4B), suggesting that Cav3.2 channels do not augment HCN1 trafficking. In addition, the activation kinetics of HCN1 channels before and after Cav3.2 channel opening were examined by fitting the rising phase of \(I_h\) with a mono-exponential function. This kinetic analysis showed that the activation time constant of HCN1 remained unaltered (P1: 50 ± 3.8 ms, P2: 55 ± 5.8 ms, Fig. 4C, p > 0.05). It is known that the HCN channel is potently modulated by

![Figure 4](image-url)
cAMP. To test whether there might be a synergistic role of Ca\(^{2+}\) and cAMP on \(I_h\), 0.5 mM Dibutyryl-cAMP (Sigma, D0627), a cell-permeable cAMP analog, was applied to the bath before testing the effects of Cav3.2 mediated Ca\(^{2+}\) influx. While delivery of cAMP increased HCN1 amplitude as expected (not shown), calcium influx via Cav3.2 did not further augment \(I_h\) amplitude (Fig. 4D, p > 0.05).

**Discussion**

It is known that Cav3 and HCN conductances interact during the process of “rebound bursting.” Several lines of evidence further showed that Cav3 channels may be functionally associated with HCN channels, but the underlying molecular details had not been explored. Here, we have identified a biochemical signaling complex between HCN1 and Cav3.2 channels in both native tissue and heterologous expression systems, showing that HCN1 channels robustly regulate Cav3.2 channel activity.

Our results revealed that the HCN1 channel forms a molecular complex with Cav3.2, but not with Cav3.1 and Cav3.3, in mouse brain tissue. This is consistent with what was previously found in cortical layer III pyramidal neurons where a colocalization of both channels was observed. We also found that coexpression of HCN1 decreased Cav3.2 current density, increased the inactivation time constant of Cav3.2, and shifted the half-activation voltage to slightly more depolarized potentials. It has been reported that HCN1 channels colocalize with and functionally regulate T-type channels in the entorhinal cortical layer III presynaptic terminals to regulate presynaptic calcium release. A detailed analysis revealed that a reduction in HCN activity resulted in a gain of function in synaptic T-type channel currents. Similar effects of \(I_h\) on T-type channel activity have been demonstrated for dendrites of hippocampal CA1 pyramidal neurons in a recent study. These findings support the notion of a functional interplay between \(I_h\) and T-type currents in the mammalian central nervous system. One key difference between these studies and our work is that we examined the effects of HCN1 on Cav3.2 channel functions under conditions in which HCN1 was blocked by cesium ions. We can thus not rule out the possibility that Cav3.2 channel activity might be further modulated in physiologic saline where sodium is able to enter via HCN1. Notwithstanding this detail, our findings showing an effect of HCN1 coexpression on Cav3.2 current density and gating may help explain the reported crosstalk between these 2 channels in neurons.

A combination of mechanisms may underlie the various effects of HCN1 on Cav3.2 channel activity. Expression of HCN1 may induce a decrease in the membrane expression of Cav3.2 channels, thus reducing the current density of Cav3.2 channels. This could occur at either the transcriptional or translational levels, or as a result of altered channel trafficking. Alternatively, the effect of HCN1 may be due to a regulation of either single channel conductance or maximum open probability of Cav3.2. Further experiments, such as surface protein biotinylation and/or single channel recordings may shed further light on this issue. A direct effect on channel function is supported by the observation that the half-activation potential and the time course of inactivation of Cav3.2 were altered. While the effect on activation gating is too small to account for the reduced whole cell current amplitude, it does suggest that the physical interaction between the channels impacts Cav3.2 activity beyond a possible effect on cell surface expression.

A number of interactions between Cav3 and other types of ion channels have been reported in the literature, including SK, IK, BK and Kv4 potassium channels. The channel structural determinants of these interactions have been delineated and shown to involve either the C-terminus region of Cav3 (Kv4), or transmembrane regions (BK). Here, we report an interaction that involves the N-terminus region of Cav3.2. This region has, to our knowledge, not previously been implicated in Cav3 channel function or trafficking, and it remains unclear whether this region regulates channel function beyond serving as an anchor for HCN1. For Kv4 channels and calcium-activated potassium channels, the association with Cav3 was shown to confer a potent calcium regulation on potassium channel function. For HCN1, our data indicate that this does not appear the case, at least not in expression systems. Unlike Kv4 channels which interact with KChIPs and calcium-activated potassium channels which either contain calcium sensors, or are conjugated to calmodulin, there appear to be no such processes in place for calcium regulation of HCN channels. Nonetheless, we note that our data are somewhat at odds with previous studies reporting a calcium-dependent upregulation of \(I_h\) current activity.
in neurons.\textsuperscript{16,17} In our experiments, it is possible that T-type calcium channel-mediated calcium entry evoked by a single membrane depolarization is simply insufficient to provide enough calcium to alter HCN channel activity, and that prolonged burst firing of neurons may result in a larger T-type channel mediated rise in intracellular calcium that then may be sufficient to modulate HCN channel function. Alternatively, there may be an indirect effect of a calcium-dependent signaling cascade that is present in neurons, but not expressed at sufficiently high levels in a heterologous system such as tsA-201 cells. Given the important role of HCN channels in regulating the neuronal excitability as well as dendritic integration, it is also possible that primary role of Cav3.2-HCN1 complexes is to modulate channel expression and trafficking to specific subcellular loci such as dendrites, rather than providing a mechanism by which T-type channels may functionally alter $I_h$. In this context, it is worth noting that functional interactions between $I_h$ and Cav3.2 calcium channels have been observed in thalamic neurons in Genetic Absence Epilepsy Rats from Strasbourg (GAERS).\textsuperscript{26} These rats carry a gain-of-function mutation in Cav3.2 channels that appears to be accompanied by an upregulation of HCN1 and HCN3 channels,\textsuperscript{26} thus highlighting a possible cross-talk between Cav3.2 channel activity and HCN channel expression.

In summary, we demonstrate a physical interaction between HCN1 and Cav3.2 channels that alters Cav3.2 channel behavior. This in turn may result in altered firing and synaptic properties of neurons that coexpress these 2 channel types.

**Materials and methods**

** Constructs**

Mouse HCN1 cDNA was a gift from Dr. Eric Accili (University of British Columbia), and the human wild-type GFP-tagged Cav3.2 intracellular linker constructs were gifts from Dr. Nobert Weiss (Academy of Sciences of the Czech Republic) and were described previously.\textsuperscript{14} The mouse HCN1 N- (amino acid residues 1–135) and C- (amino acid residues 382–908) termini were amplified by PCR, followed by sequencing and subcloning into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA); the mKate2 tag was amplified and inserted upstream of HCN1 fragments.

The primers used for HCN1 N-terminus were 5′ cgggatccATGGAAGGCGGGCCAAACCC 3′ and 5′ccaccggtTCAATCCCAATAAACCTGAAGTC3′, for HCN1 C-terminus were 5′cgggatccGCCACAGCTTTGATCCAGTCT3′ and 5′ccaccggtTCAATAATTCGAAAGCAACG3′. The primers used for amplifying mKate2 sequence were 5′ cgggatccGCCACCATGGTGAGCGAGCT 3′ and 5′ cgggatccTCTGAGTCCGGAACCTCCTC3′.

**Transient transfection**

TsA-201 cells were cultured and transiently transfected as described previously.\textsuperscript{9} Briefly, cells were plated on 10 cm plates with or without glass coverslips and transfected using the calcium phosphate method using the following combination of cDNAs: for electrophysiology, 3 μg of Cav3.2 (or empty vector pcDNA3.1), 1 μg of HCN1 (or empty vector pcDNA3.1) and 0.5 μg of pEGFP were transfected. For the co-immunoprecipitation experiments to map the interaction site on HCN1: 1 μg of either full length HCN1, mKate-tagged HCN1 N-terminus or mKate-tagged HCN1 C-terminus and 3 μg of Cav3.2 were transfected. To map the interaction site on Cav3.2: 3 μg of either full length Cav3.2, GFP-tagged Cav3.2 N-terminus, GFP-tagged Cav3.2 C-terminus or GFP-tagged Cav3.2 intracellular linkers and 1 μg of full length HCN1 cDNAs were transfected. Cells were incubated for 72 hours after transfection and before experiments.

**Co-Immunoprecipitation and western blotting**

Mouse brain proteins were extracted in RIPA buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 0.5 mM PMSF and protease inhibitor cocktail) and 1 mg of lysates was pre-cleared with 30 μl of Protein G Sepharose beads (GE Healthcare) for 2 hours at 4°C. Samples were then centrifuged and supernatants were incubated with Protein G beads and 2 μg of one of the following antibodies: anti-Cav3.1 (Alomone, ACC-021), anti-Cav3.2 (Alomone, 025), anti-Cav3.3 (Alomone, ACC-0009) or an irrelevant antibody, overnight, at 4°C, with rotation. Beads were washed 3 times with modified RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS) and twice with PBS, and eluted with 2 X Laemmli sample buffer.
For the co-immunoprecipitation experiments using heterologous expression, cultured tsA-201 cells were transiently transfected as described above. Seventy-two hours after transfection, cells were lysed in modified RIPA buffer on ice, for 1 hour. Lysates were transferred to micro centrifuge tubes and centrifuged at 13,000 RPM for 20 min to clear debris. Supernatants were then incubated overnight at 4°C with 2 μg of either anti-GFP (Santa Cruz, sc-8334) or anti-tRFP (against mKate-2, Evrogen, AB-233). After incubation with antibodies, Protein A Sepharose beads were added to the samples and incubated at 4°C for 1.5 hours. Beads were washed 3 times with modified RIPA buffer and proteins were eluted with 2 X Laemmli sample buffer.

Eluted samples were loaded on the appropriate percentage Tris-glycine gel and resolved using SDS-PAGE. Samples were transferred to a 0.2 μm nitrocellulose membrane (Bio-Rad) and western blot analysis was performed using one of the following antibodies: anti-HCN1 (1:300, Neuromab, 75–110), anti-HCN2 (1:300, Neuromab, 71–37), anti-Cav3.1 (1:200, Alomone, ACC-021), anti-Cav3.2 (1:200, Santa Cruz, sc-25691), and anti-Cav3.3 (1:200, Alomone, ACC-0009). Horseradish peroxidase-linked secondary anti-mouse and anti-rabbit antibodies were used at a 1:5,000 dilution and blots were developed using a C-DiGit blot scanner (LI-COR Biosciences).

**Electrophysiology**

Whole-cell voltage-clamp recordings were made from cultured tsA-201 cells at room temperature using an Axopatch 200B amplifier (Axon Instruments). The external solution for Cav3.2 recordings contained (mM): 125 CsCl, 10 BaCl2, 1 MgCl2, 10 D-glucose and 10 HEPES (adjusted to pH 7.4 with CsOH). The intracellular pipettes were pulled from borosilicate glass (with an impedance of 3–5 MΩ) and filled with an intracellular solution containing (mM): 140 CsCl, 2.5 CaCl2, 1 MgCl2, 5 EGTA, 2 Na-ATP, 0.3 Na-GTP, and 10 HEPES (adjusted to pH 7.3 with CsOH). The external solution for HCN recordings contained (mM): 150 NaCl, 5 KCl, 2.5 CaCl2, 1 MgCl2, 10 HEPES and 10 D-glucose (adjusted to pH 7.4 with NaOH). The internal contained (mM): 140 KGlucunate, 4 Mg-ATP, 0.5 Na-GTP, 0.5 EGTA and 10 HEPES (adjusted to pH 7.2 with KOH).

$I-V$ curves were fitted with the following Boltzmann equation:

$$I = \frac{G_{\text{max}} \times (V_{m} - V_{r})}{1 + \exp\left(\frac{-(V_{m} + V_{0.5\text{act}})}{k}\right)}$$

where $I$ is the normalized current by the cell capacitance, $G_{\text{max}}$ is the value of maximal conductance, $V_{m}$ is the membrane potential, $V_{r}$ is the reversal potential, $V_{0.5\text{act}}$ is the membrane potential for the half-activation and $k$ is the slope factor. The conductance was calculated according to the equation: $G = I/(V_{m} - V_{r})$. The reversal potential of barium currents determined by the mean value of individual current fittings was 36.1 ± 0.9 mV (without HCN1) and 32.1 ± 1.5 mV (with HCN1). Steady-state inactivation curves were fitted with the modified Boltzmann equation:

$$\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp\left(\frac{-(V_{p} - V_{0.5\text{inact}})}{k}\right)}$$

where $I/I_{\text{max}}$ is the normalized current, $V_{p}$ is the conditioning prepulse, $V_{0.5\text{inact}}$ is the voltage for half-inactivation.

HCN currents were leak-subtracted using a $p/4$ subtraction protocol. Series resistance in voltage-clamp recordings was compensated 50–70% and continually monitored through experiments. Recordings were terminated whenever significant increases (≥ 20%) in access resistance occurred. Current signals were filtered at 2 kHz (Digidata 1320A, Molecular Devices), respectively, in all experiments. Clampex 9.2 software running on a computer was used to acquire data.

**Statistical analysis**

Statistical analyses were performed using Origin9 and Sigmaplot10.0. Data are expressed as means ± SEM. Statistical analyses were done using 2-tailed unpaired Student’s $t$-tests. Significance was set at $p < 0.05$.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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Author contributions

J.F. and G.W.Z. designed the study and wrote the manuscript. J.F., M.A.G., F.X.Z., L.C., I.A.S. performed experiments. J.F. performed data analysis. G.W.Z. supervised the study.

References

[1] Bourinet E, Altier C, Hildebrand ME, Trang T, Salter MW, Zamponi GW. Calcium-permeable ion channels in pain signaling. Physiol Rev 2014; 94(1):81-140; PMID:24382884; https://doi.org/10.1152/physrev.00023.2013

[2] Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev 2015; 67(4):821-70; PMID:26362469; https://doi.org/10.1124/pr.114.009654

[3] Tsay D, Dudman JT, Siegelbaum SA. HCN1 channels constrain synaptically evoked Ca2+ spikes in distal dendrites of CA1 pyramidal neurons. Neuron 2007; 56 (6):1076-89; PMID:18093528; https://doi.org/10.1016/j.neuron.2007.11.015

[4] 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 (4):478-86; PMID:21358644; https://doi.org/10.1038/nn.2757

[5] Magee JC, Christofi G, Miyakawa H, Christie B, Lasser-Ross N, Johnston D. Subthreshold synaptic activation of voltage-gated Ca2+ channels mediates a localized Ca2+ influx into the dendrites of hippocampal pyramidal neurons. J Neurophysiol 1995; 74(3):1335-42; PMID:7500154

[6] Swensen AM, Bean BP. Ionic mechanisms of burst firing in dissociated Purkinje neurons. J Neurosci 2003; 23 (29):9650-63; PMID:14573545

[7] Engbers JD, Anderson D, Tadayonnejad R, Mehaffey WH, Mol ineux ML, Turner RW. Distinct roles for I(T) and I(H) in controlling the frequency and timing of rebound spike responses. J Physiol 2011; 589(pt22):5391-413; PMID:21969455; https://doi.org/10.1113/jphysiol.2011.215632

[8] Kole MH, Hallermann S, Stuart GJ. Single I\(h\) channels in pyramidal neurondendrites: properties, distribution, and impact on action potential output. J Neurosci 2006; 26 (6):1677-87; PMID:16467515; https://doi.org/10.1523/JNEUROSCI.3664-05.2006

[9] Rehak R, Bartoletti TM, Engbers JD, Berecki G, Turner RW, Zamponi GW. Low voltage activation of Cav3.1 channels. PLoS One 2013; 8 (4):e61844; PMID:23626738; https://doi.org/10.1371/journal.pone.0061844

[10] McCormick DA, Pape HC. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol 1990; 431:291-318; PMID:1712843; https://doi.org/10.1113/jphysiol.1990.sp018331

[11] Sangrey T, Jaeger D. Analysis of distinct short and prolonged components in rebound spiking of deep cerebellar nucleus neurons. Eur J Neurosci 2010; 32(10):1646-57; PMID:21039958; https://doi.org/10.1111/j.1460-9568.2010.07408.x

[12] Kaku T, Lee TS, Arita M, Hadama T, Ono K. The gating and conductance properties of Cav3.2 low-voltage-activated T-type calcium channels. Jpn J Physiol 2003; 53 (3):165-72

[13] Talavera K, Nilius B. Biophysics and structure-function relationship of T-type Ca\(2+\) channels. Cell Calcium 2006; 40(2):97-114; PMID:16777221; https://doi.org/10.1016/j.cellc.2006.04.013

[14] Rzhepetskyy Y, Lazniewska J, Prof J, Campiglio M, Flucher BE, Weiss N. A Cav3.2/Stacl molecular complex controls T-type channel expression at the plasma membrane. Channels (Austin) 2016; 10(5):346-54; PMID:27149520; https://doi.org/10.1080/19336950.2016.1186318

[15] Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 2003; 65:453-80; PMID:12471170; https://doi.org/10.1146/annurev.physiol.65.092101.142734

[16] Luthi A, McCormick DA. Periodicity of thalamic synchronized oscillations: the role of Ca\(2+\)-mediated upregulation of I\(h\). Neuron 1998; 20(3):553-63; PMID:9539128; https://doi.org/10.1016/S0896-6273(00)80994-0

[17] Luthi A, McCormick DA. Ca\(2+\)-mediated up-regulation of I\(h\) in the thalamus. How cell-intrinsic ionic currents may shape network activity. Ann N Y Acad Sci 1999; 868:765-9; https://doi.org/10.1111/j.1749-6632.1999.tb11354.x

[18] Ingram SL, Williams JT. Modulation of the hyperpolarization-activated current (I\(h\)) by cyclic nucleotides in guinea-pig primary afferent neurones. J Physiol 1996; 492(Pt1):97-106; https://doi.org/10.1113/jphysiol.1996.sp021292

[19] Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA, Tibbs GR. Molecular mechanism of cAMP modulation of HCN pacemaker channels. Nature 2001; 411 (6839):805-10; https://doi.org/10.1038/35081088

[20] Cueni L, Canepari M, Lujan R, Emmenegger Y, Watanabe M, Bond CT, Franken P, Adelman JP, Luthi AT-type Ca\(2+\) channels, SK2 channels and SERCA
gate sleep-related oscillations in thalamic dendrites. Nat Neurosci 2008; 11(6):683-92; https://doi.org/10.1038/nn.2124

[21] Engbers JD, Anderson D, Asmara H, Rehak R, Mehaffe WH, Hameed S, Mckay BE, Kruskic M, Zamponi GW, Turner RW. Intermediate conductance calcium-activated potassium channels modulate summation of parallel fiber input in cerebellar Purkinje cells. Proc Natl Acad Sci U S A 2012; 109(7):2601-6; https://doi.org/10.1073/pnas.1115024109

[22] Engbers JD, Zamponi GW, Turner RW. Modeling interactions between voltage-gated Ca\(^{2+}\) channels and KCa1.1 channels. Channels (Austin) 2013; 7(6):24-5; https://doi.org/10.4161/chan.25867

[23] Anderson D, Mehaffe WH, Iftinca M, Rehak R, Engbers JD, Hameed S, Zamponi GW, Turner RW. Regulation of neuronal activity by Cav3-Kv4 channel signaling complexes. Nat Neurosci 2010; 13(3):333-7; https://doi.org/10.1038/nn.2493

[24] Anderson D, Rehak R, Hameed S, Mehaffe WH, Zamponi GW, Turner RW. Regulation of the K(V)4.2 complex by Ca(V)3.1 calcium channels. Channels (Austin) 2010; 4(3):163-7; https://doi.org/10.4161/chan.4.3.11955

[25] Faber ES, Sah P. Calcium-activated potassium channels: multiple contributions to neuronal function. Neuroscientist 2003; 9(3):181-94; https://doi.org/10.1177/1073858403009003011

[26] Cain SM, Tyson JR, Jones KL, Snutch TP. Thalamocortical neurons display suppressed burst-firing due to an enhanced Ih current in a genetic model of absence epilepsy. Pfluegers Arch 2015; 467(6):1367-82; https://doi.org/10.1007/s00424-014-1549-4