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HCN channels in developing neuronal networks

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Abstract

Developing neuronal networks evolve continuously, requiring that neurons modulate both their intrinsic properties and their responses to incoming synaptic signals. Emerging evidence supports roles for the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in this neuronal plasticity. HCN channels seem particularly suited for fine-tuning neuronal properties and responses because of their remarkably large and variable repertoire of functions, enabling integration of a wide range of cellular signals. Here, we discuss the involvement of HCN channels in cortical and hippocampal network maturation, and consider potential roles of developmental HCN channel dysregulation in brain disorders such as epilepsy.

1. Molecular and functional characteristics of HCN channels

1.1. I_h and its functions in neurons and neuronal networks

The hyperpolarization-activated current, I_h , originally observed in motoneurons (Araki et al., 1961) and later characterized in heart (Noma and Irisawa, 1976) and retinal photoreceptors (Fain et al., 1978), has long puzzled scientists, because of its unorthodox physiological behavior which led to its nicknaming as f- (funny) or q- (queer) current (Brown and DiFrancesco, 1980; Halliwell and Adams, 1982; Pape, 1996).

I_h is a slow, non-inactivating conductance that is activated by hyperpolarization to potentials negative to- or close to typical neuronal resting membrane potential (Figure 1). This permits Na^+ and K^+ entry, providing the basis of the fundamental biophysical function of this conductance. First, tonic activation of I_h helps set the resting membrane potential at a somewhat depolarized level (Maccaferri et al., 1993; Lupica et al., 2001; Nolan et al., 2003; Meuth et al., 2006). Second, I_h reduces input resistance, so that the influence of any current on membrane potential is reduced (as per $V = I \times R$; Magee, 1998, 1999; Nolan et al., 2004; Surges et al., 2004). Together with the selective subcellular localization of I_h -conducting HCN channels, these basic properties lead to opposing effects of I_h on neuronal excitability, that are governed also by type of HCN channel isoform, and the type of input reaching the cell (Santoro and Baram, 2003; Dyhrfjeld-Johnsen et al., 2008). Thus, elegant studies (Magee, 1998, 1999; Williams and Stuart, 2000, 2003; Berger et al., 2001, 2003; Poolos et al., 2002, 2006; Wang et al., 2003; Fan et al., 2005; van Welie et al., 2006; Brager and Johnston, 2007) have

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demonstrated that dendritic I_h reduces neuronal excitability by reducing membrane resistance and dendritic summation in response to dendritic depolarizing input. In contrast, increased neuronal excitability with increase of I_h has been reported, because this current depolarizes resting membrane potential (Chen K. et al., 2001), driving the neuron closer to firing threshold of action potentials (Chen K. et al., 2001; Dyhrfjeld-Johnsen et al., 2008). Therefore, an emerging consensus view suggests that I_h is crucial for intrinsic neuronal excitability (Zhang and Linden, 2003; Beck and Yaari, 2008). In addition, the final influence of I_h on neuronal excitability is governed by a balance of opposing actions on resting membrane potential and resistance properties. In a non-firing cell, these actions of the h-current exert a stabilizing effect on the membrane potential (Maccaferri et al., 1993; Lupica et al., 2001; Nolan et al., 2003; Meuth et al., 2006; Figure 1C). They also influence rhythmicity of firing (Pape, 1996; Lüthi and McCormick, 1998; Fisahn et al., 2002; Cobb et al., 2003; Figure 1B) and resonance behavior (Magee, 1999; Ulrich, 2002; Nolan et al., 2004; Bernard et al. 2007; Narayanan and Johnston, 2007).

1.2. The molecular basis of I_h : a key to the diverse functions of this conductance

The characterization of four genes that encode I_h -generating channel molecules has shed light on the unique properties of this current (Santoro et al., 1997; Gauss et al., 1998; Ludwig et al., 1998; Santoro et al., 1998; Ishii et al., 1999). These molecular studies revealed that I_h is generated by a nonselective cation channel named HCN (Hyperpolarization-activated Cyclic Nucleotide-gated channel; Clapham, 1999). The four known isoforms (HCN1-4) can assemble into homo- or heteromeric channels with different activation kinetics, voltage dependence and cAMP-sensitivity (Kaupp and Seifert, 2001; Much et al., 2003; Robinson and Siegelbaum, 2003; Santoro and Baram, 2003; Brewster et al., 2005; Figure 1A), thus generating a broad repertoire of sensitivity to the neuronal environment, as well as a large spectrum of biophysical channel properties.

In mammalian brain, the expression patterns of HCN channel isoforms are distinct but partially overlapping (Moosmang et al., 1999; Monteggia et al., 2000; Santoro et al., 2000; Bender et al., 2001; Notomi and Shigemoto, 2004). Thus, individual neurons can express more than one HCN channel isoform (Franz et al., 2000; Santoro et al., 2000; Brewster et al., 2002; Brewster et al., 2007a), and the I_h current of such neurons reflects the properties of the contributing isoforms (Franz et al., 2000; Santoro et al., 2000; Vasilyev and Barish, 2002; Santoro and Baram, 2003; Surges et al., 2006). These properties may reflect the mathematical sum of primarily homomeric channels, may result from the formation of heteromeric channels deriving from interaction among the different isoforms expressed in an individual neuron, or result from the combination of both homomeric and heteromeric channels in the neuron (Santoro et al., 2000; Ulens and Tytgat, 2001; Chen S. et al., 2001; Simeone et al., 2005a; Simeone et al., 2005b; Dekker and Yellen, 2006; Kole et al., 2006; Budde et al., 2008). In heterologous systems, promiscuous heteromerization of almost all HCN channel isoforms has been described (Chen S. et al., 2001; Proenza et al., 2002; Altomare et al., 2003; Much et al., 2003; Whitaker et al., 2007), and evidence is increasing that heteromeric channels, with properties differing from the properties of pure homomeric channels (Chen S. et al., 2001; Ulens and Tytgat, 2001), also play a role in brain *in vivo* (Brewster et al., 2005; Kuisle et al., 2006; Budde et al., 2008). However, in rodent hippocampus, heteromerization seems to be restricted, and is governed by network activity (Much et al., 2003; Brewster et al., 2005; Brewster et al., 2007b; Zha et al., 2008).

Interestingly, for channels that function primarily at subthreshold membrane potential levels, HCN channels are endowed with remarkable plasticity of function. This plasticity involves short-term regulation of channel activity through cellular metabolites (DiFrancesco and Tortora, 1991; Wang et al., 2002; van Welie et al., 2004; Simeone et al., 2005b; Pian et al.,

2006; Zolles et al., 2006; Fogle et al., 2007; Pian et al., 2007) or phosphorylation (Zong et al., 2005; Poolos et al., 2006) as well as longer-lasting modulation via regulation of expression (Bräuer et al., 2001; Brewster et al., 2002, 2005), heteromerization (Proenza et al., 2002; Altomare et al., 2003; Much et al., 2003; Brewster et al., 2005; Whitaker et al., 2007; Zha et al., 2008) or subcellular transport of channel isoforms (Bender et al., 2007; Brewster et al., 2007a; Shin and Chetkovich, 2007). A potential association of HCN channel isoforms with β -subunits or scaffolding proteins may add further variety to the channels' functions (Gravante et al., 2004; Kimura et al., 2004; Qu et al., 2004; Santoro et al., 2004). Thus, rather than functioning solely as 'membrane stabilizers', HCN channels are in an excellent position to fine-tune a neuron's responses to external stimuli by integrating a wide range of cellular signals. In fact, essential brain functions such as theta resonance underlying learning and memory (Nolan et al., 2004; Wang et al., 2007), thalamic rhythm generation (Ludwig et al., 2003; Budde et al., 2005; Kuisle et al., 2006) or the subcortical control of motor behavior (Nolan et al., 2003; Chan et al., 2004) may depend on this property of the HCN channels.

Recent work suggests that the properties of the HCN channels highlighted above are particularly important during neuron and network development. Maturation requires developing neurons to modulate both their intrinsic properties as well as their firing behaviors to a constantly changing neuronal milieu. Ion channels such as the HCN channels, that are endowed with high plasticity of function may be particularly suited for this process (Zhang and Linden, 2003; Beck and Yaari, 2008). Consistent with this notion, an age-dependence of the properties of I_h , suggesting age-specific roles of this current, has been described in several brain regions (Bayliss et al., 1994; Vasilyev and Barish, 2002, 2004; Tanaka et al., 2003; Bajorat et al., 2005; Surges et al., 2006).

Thus, a significant body of work has focused on this current and its building blocks, the HCN channels, over the past few years, contributing to an increasing understanding of the role of HCN channels in normal and pathological neuronal and network activities (Robinson and Siegelbaum, 2003; Santoro and Baram, 2003; Frere et al., 2004; Herrmann et al., 2007; Dyhrfjeld-Johnsen et al., 2008; Richichi et al., 2008). This information, and specifically the new perspectives on the role of I_h in developing hippocampal and cortical networks, is the focus of the following paragraphs.

2. Developmental aspects of HCN channels

2.1. The transition from immature to mature network activity involves changes in HCN isoform expression levels

In hippocampus, patterns of network activity depend, at least in part, on the expression levels and specific combinations of HCN isoforms that are present in certain neuronal populations at a given developmental period. For instance, in hippocampal area CA3, I_h may contribute critically to age-specific hippocampal network activity. The principal network activity in neonatal CA3 is low-frequency (0.1–0.3 Hz), synchronized population discharges (intracellularly seen as “giant depolarizing potentials”, GDPs) that are believed to contribute to network differentiation (Ben-Ari, 2002). Importantly, CA3 pyramidal cells govern the generation of GDPs, which are driven by spontaneous burst activity originating from these cells (Sipilä et al., 2005). The capacity of these neurons to generate these spontaneous bursts is reduced when HCN channels are blocked, resulting in disruption of GDP generation (Bender et al., 2005). This indicates that HCN channel activity may be crucial for network synchronization in neonatal CA3 (Bender et al., 2005; but see Sipilä et al., 2006). At the molecular level, HCN isoforms 1, 2 and 4 (and low levels of HCN3) are expressed in area CA3, in both principal cells and interneurons (Moosmang et al., 1999; Santoro et al., 2000; Bender et al., 2001; Notomi & Shigemoto, 2004). Expression commences as early as postnatal day 2 (Bender et al., 2001; Vasilyev and Barish, 2002; Bender et al., 2005; Brewster et al., 2007a),

and the number of cells expressing HCN channels is highest during the neonatal period (Bender et al., 2005). Remarkably, I_h -current density also peaks during the first postnatal week in CA3 pyramidal cells and declines thereafter (Vasilyev and Barish, 2002), suggesting that HCN expression may underlie the age-specific function of the current and is regulated to enable the synchronizing function of I_h . Related findings, demonstrating a modulating effect of I_h on the regularity of burst firing in neonatal CA3 (Agmon and Wells, 2003), further support this notion.

Developmental changes of HCN channel expression also occur in CA1. In CA1 pyramidal cells, quantitative changes in HCN1, HCN2 and HCN4 isoform expression constitute a “molecular switch” from an early-postnatal preponderance of slow-activating, cAMP-sensitive isoforms (HCN2 and 4) to a mature stage in which the major contribution to I_h -properties comes from the fast-activating, cAMP-insensitive HCN1 isoform (Surges et al., 2006; Brewster et al., 2007a). Interestingly, the time course of these changes correlates with the developmental transition from the slow network oscillations that prevail in immature CA1 towards mature network activity, including theta frequency (LeBlanc and Bland, 1979; Garaschuk et al., 1998). The HCN expression changes may contribute to this transition via modulation of neuronal resonance behavior (Magee, 1998; Hutcheon and Yarom, 2000; Ulrich, 2002; Narayanan and Johnston, 2007). Thus, with I_h -activation accelerating during development (Surges et al., 2006), pyramidal cells may get closer to their preferred (mature) firing frequency (Pike et al., 2000; Ulrich, 2002; Bernard et al., 2007).

2.2. Subcellular distribution of HCN channels: Developmental patterns and implications

The functions of the HCN channels depend not only on their expression levels, but also on their sub-cellular localization (Magee, 1998; Poolos et al., 2002; Santoro and Baram 2003; Williams and Stuart, 2003; Berger et al., 2003; Aponte et al., 2006; Bender et al., 2007; Ying et al., 2007). This localization can vary significantly among neuronal types and may include somatic and dendritic, as well as axonal compartments (Figure 2). For instance, in cortical and hippocampal pyramidal cells, HCN channels localize preferentially to dendrites, where they control the integration and summation of synaptic input (Magee, 1998, 1999; Stuart and Spruston, 1998; Williams and Stuart, 2000, 2003; Berger et al., 2001, 2003; Poolos et al., 2002; Lörincz et al., 2002; Wang et al., 2003; Notomi and Shigemoto, 2004; Brewster et al., 2007a). In cortical and hippocampal interneurons, these channels are often found in the somatic compartment, where they regulate cell properties such as the resting membrane potential (Maccferri and McBain, 1996; Lupica et al., 2001). Axonal HCN channels have been identified in a subgroup of interneuron populations (Notomi and Shigemoto, 2004; Lujan et al., 2005; Aponte et al., 2006; Brewster et al., 2007a), as well as in certain excitatory neurons (Cuttle et al., 2001; Soleng et al., 2003; Bender et al., 2007). In these neurons, the channels localize predominantly to axon terminals, where they seem to modulate neurotransmitter release (Southan et al., 2000; Klar et al., 2003; Aponte et al., 2006; Bender et al., 2007).

2.2.1. Developmental regulation and age-specific functions of axonal HCN channels

Recent data suggest that the sub-cellular localization of HCN channels is also subject to developmental regulation. For example, in rodent perforant path, the major afferent pathway to the hippocampus, HCN1 channel isoforms are expressed in axon terminals that abut on dendrites of the dentate gyrus granule cells (Figure 3). Interestingly, this expression is specific to both an age window and the type of axons involved: in the perforant path, which can be subdivided into a medial and a lateral portion, only axons of the medial portion (medial perforant path, mPP) express HCN1 channels, whereas the axons of the lateral perforant path, that originate in a separate neuronal population in the entorhinal cortex (Amaral and Witter, 1995), are devoid of this channel. In addition, HCN1 channel expression is robust in immature mPP, and gradually decreases with maturation (Figure 3A–D). As described above for area CA3, the age-specific subcellular localization of the HCN1 channels to mPP axons is associated

with a specific developmental *function* of I_h : the current prevents a variant of short-term depression (STD), promoting synaptic strengthening. Thus, in a stimulation paradigm that determines STD of mPP firing, a form of synaptic plasticity that is attributable to presynaptic mechanisms (Harris and Cotman, 1985; Dietrich et al., 1997), blocking I_h caused an increase of STD in immature but not in mature mPP, suggesting that synaptic properties in mPP are modulated by HCN channel activity in an age-dependent manner (Figure 3E). These findings raise the possibility that pre-synaptic I_h might enhance the probability and reliability of neurotransmitter release at immature mPP synapses, an effect that might involve a tonic depolarization of the presynaptic membrane potential (Beaumont and Zucker, 2000; Southan et al., 2000; Mellor et al., 2002; Agmon and Wells, 2003; Aponte et al., 2006). Because reliability of neuronal firing is a prerequisite for stabilization of synaptic contacts at perforant path/granule cell synapses (Frotscher et al., 2000), the expression of HCN1 specifically in axon terminals of immature mPP can be seen as a contribution of the channels to the maturation of network connectivity.

2.2.2. Developmental regulation and age-selective function of dendritic HCN channels—

Developmental regulation of the sub-cellular distribution of the HCN channels has been described also for the dendritic compartment. In mature rat hippocampus, dendrites of CA1 pyramidal cells express channels of both the HCN1- and HCN2-type (Lörincz et al., 2002; Notomi and Shigemoto, 2004; Brewster et al., 2007a), whereas HCN4 type channels are barely detectable in these dendritic domains. In contrast, during the first two postnatal weeks, only HCN1 channels localize to these dendrites, whereas HCN2 channels, although expressed in the soma neonatally, are only detectable in the dendrites starting in the third postnatal week (Brewster et al., 2007a). These findings predict that dendritic I_h in the immature hippocampus would be comprised of homomeric HCN1 channels, and would possess biophysical properties that differ from those of adult-type dendritic I_h . The developmental trigger for this delayed dendritic transport of HCN2 channels is not yet known. Developmental transitions in hippocampal network activity that occur during this developmental period might be involved (see above). Alternatively, isoform-specific interaction between HCN channel isoforms and chaperone/transport-related proteins may be responsible (e.g., Gravante et al., 2004; Kimura et al., 2004; Santoro et al., 2004; Shin et al., 2008). Developmental expression profiles of individual interacting proteins would then govern the trafficking of individual HCN channels to dendrites and an onset of this trafficking. Finally, in heterologous systems, heteromerization might contribute to transport of HCN channels to the membrane (Proenza et al., 2002; Altomare et al., 2003; Much et al., 2003; Whitaker et al., 2007). Stable heteromerization of HCN1/HCN2 involves mature glycosylation of the HCN1 isoform, and may be age-dependent (Brewster et al., 2007b; Zha et al., 2008), potentially influencing the transport of HCN2 to the dendritic compartment early in life. Because the function of dendritic HCN channels in dendritic summation and neuronal excitability is crucial for network stability (Magee, 1998, 1999; Berger et al., 2001; Williams and Stuart, 2003; Wang et al., 2003; van Welie et al., 2004; Fan et al., 2005; Brager & Johnston, 2007; Tsay et al., 2007), further examination of the unique developmental mechanisms determining HCN1 and HCN2 channel trafficking to the dendrites, and their contribution to dendritic I_h , is important. It is tempting to speculate that dendritic I_h conducted by homomeric HCN1 channels would have properties (e.g., rapid kinetics, minimal response to fluctuating cAMP levels) that promote selective stabilization of neuronal connectivity by influencing dendritic summation to the developing neuronal somata (Magee, 1999).

3. The role of neuronal activity in developmental HCN regulation

3.1. Neuronal activity is a driving force for developmental HCN expression changes

The findings summarized above demonstrate a remarkable evolution of the expression and subcellular localization of the HCN channels during the early-postnatal period in hippocampus. This raises the question of the regulation of these changes. Generally, the developmental organization of neuronal networks is governed by genetic as well as environmental elements. Among the latter, neuronal activity has long been recognized to be a key determinant (Goodman and Shatz, 1993; Constantine-Paton and Cline, 1998), suggesting that evolving network activity may influence the developmental expression of the HCN channels as well.

The effects of neuronal activity on the expression of the HCN channels in developing hippocampus were discovered in studies employing network activity bursts (experimental seizures) induced by the glutamate agonist kainic acid and by hyperthermia (Brewster et al., 2002). These bursts provoked alteration of the expression patterns of the HCN channels that co-varied with seizure duration, as well as persistent changes in the biophysical properties of I_h . Thus, the abnormal activity bursts shifted the voltage dependence of I_h in the depolarizing direction and caused a slowing of its activation and deactivation kinetics – changes that resulted in a generally larger h-current in hippocampal CA1 pyramidal cells (Chen K. et al., 2001; Kamal et al., 2006). These changes persisted for months and, together with the observed increase of perisomatic IPSCs (Chen et al., 1999), led to increased rebound firing and hyperexcitability in the hippocampal network (Dube et al., 2000, 2006; Chen K. et al., 2001). The molecular basis for these physiological changes consisted of reduced expression of the fast-activating and -deactivating HCN1 isoform, decreasing its proportional contribution to the HCN channel pool and accounting for the slowing of I_h kinetics. Decreased HCN1 protein levels also increased the stochastic probability of formation of HCN1/HCN2 heteromers (Figure 4A), and a doubling of heteromeric HCN1/HCN2 channels (Brewster et al., 2005; Zha et al., 2008). The biophysical properties of these heteromers are not fully known, and available data suggest that they may not intermediate to those of HCN1 or HCN2 homomers (Chen S. et al., 2001). It is tempting to speculate that the unusual properties of the I_h recorded after seizures (slowed kinetics, depolarized activation curve and modest cAMP response) are a result of increased contribution of heteromeric channels to the total cellular I_h . Importantly, both the molecular and physiological changes in the channels endured for months, suggesting that the pathological (seizure) activity bursts interfered with ('corrupted') the normal developmental program of HCN channel expression in hippocampus (Figure 4B).

3.2. How does network activity regulate HCN channel expression and function?

The intracellular signaling mechanisms that mediate activity-dependent changes of HCN channel expression have been partially elucidated. Using organotypic hippocampal cultures as an *in vitro* seizure model, Richichi and colleagues (2008) found that the seizure-induced reduction of HCN1 mRNA and protein expression required Ca^{2+} -influx into the bursting neurons via Ca^{2+} -permeable AMPA-receptors, followed by activation of CaM Kinase II. Analysis of the HCN1 gene promoter further revealed a highly conserved binding site for the transcription factor Neuronal Restrictive Silencing Factor (NRSF/REST), and the expression of this factor was enhanced by the network activity bursts (Richichi et al., 2007) suggesting that, further downstream of CaM Kinase II, NRSF might be involved in the pathway by which activity-bursts regulate HCN1 channel expression (Richichi et al., 2007). Interestingly, no part of this pathway was involved in the upregulation of HCN2 mRNA by seizure-like network bursts (Richichi et al., 2008), suggesting that multiple, isoform-specific signaling mechanisms contribute to activity-dependent regulation of hippocampal HCN transcription.

Network activity may also regulate the function of HCN channels via post-translational mechanisms. In the *in vitro* model described above, seizure-like network activity increased the stable heteromerization of HCN1/HCN2 (as found also *in vivo*, Brewster et al., 2005). Interestingly, the increased association of HCN1/HCN2 was not attributable solely to a stochastic phenomenon resulting from reduced HCN1 channel expression and increased probability of each HCN1 molecule's interaction with an HCN2 subunit (Figure 4A). Rather, an additional mechanism for this activity-induced heteromerization of HCN1 and HCN2 isoforms involved enhanced HCN1 channel glycosylation by network activity bursts (Zha et al., 2008), and preferential stable co-assembly of glycosylated HCN1 with the HCN2 isoforms. Thus, *in vivo* and *in vitro*, both transcriptional and post-translational regulatory mechanisms are triggered by hippocampal network activity to influence HCN expression, co-assembly and trafficking. These, in turn, govern persistent changes in the properties of I_h .

3.3. Activity governs trafficking of HCN channels to subcellular compartments

The age-specific localization of HCN channels to subcellular compartments, discussed above, has recently been found to be largely governed by age-dependent neuronal activity. For dendritic transport, Shin and Chetkovich (2007) used organotypic hippocampal slice cultures to study the developmental evolution of dendritic HCN1 expression and the mechanisms that govern it. They found that enrichment of HCN1 channels in the distal segment of CA1 pyramidal cell dendrites did not occur when neuronal activity was blocked, or when they transected the temporoammonic pathway, suggesting that neuronal activity deriving from afferent innervation is a major driving force for the transport of HCN1 channels to their proper distal dendritic location. Similar mechanisms may function in the mature hippocampus: In hippocampal slices from young adult rats that were theta-burst-stimulated to induce LTP, Fan et al. (2005) observed an activity-dependent, dynamic modulation of dendritic I_h in CA1 neurons that correlated with altered expression levels of the HCN1 subunit. However, because acute slices were used in this study, a dynamic regulation of the dendritic transport of HCN1 channels could not be demonstrated. Interestingly, both studies reported a role for CaM Kinase II-activity in regulating dendritic HCN1, concordant with the findings of Richichi et al. (2008) on the activity-dependent regulation of HCN1 transcription (see above).

Axonal distribution of HCN1 during development was also activity-dependent (Bender et al., 2007): When organotypic entorhino-hippocampal cultures were incubated for a week with tetrodotoxin (TTX), suppressing >90% of action potentials (Denac et al., 2000), this led to increased presynaptic presence of HCN1 channels. This finding is consistent with a suppressive effect of network activity on the expression of these channels in perforant path axon terminals. TTX did not affect the transcription and translation levels of HCN1 channels in the cells of axonal origin, i.e., the entorhinal cortex layer II stellate cells, indicating that the activity-dependent regulation was at the post-translational, trafficking level (Bender et al., 2007).

4. Interactions of HCN channels with other conductances

The findings described above indicate that the balance of HCN isoform expression is critical for network maturation in hippocampus, and presumably other regions of the brain. However, HCN channels are unlikely to be the only determinants of this process. In fact, the intricate interplay of I_h and other conductances in processes such as dendritic information processing in cortical neurons (Williams and Stuart, 2003; Day et al., 2005) or rhythm generation in entorhinal cortex (Dickson et al., 2000; Fransen et al., 2004) and thalamus (Lüthi and McCormick, 1998; Budde et al., 2005) suggests that establishment of network functions requires a coordinated expression program of several conductances, which may homeostatically influence one another (MacLean et al., 2003; Santoro and Baram, 2003; Misonou and Trimmer, 2004; Moody and Bosma, 2005; Meuth et al., 2006; Schulz et al., 2007; Budde et al., 2008).

Likely candidates for this process include potassium channels, for which a developmental regulation of hippocampal expression has been shown (Maletic-Savatic et al., 1995; Tinel et al., 1998; Grosse et al., 2000; Antonucci et al., 2001; Smith et al., 2001; Tansey et al. 2002; Falk et al., 2003; Kanaumi et al., 2008). Some of these channels show interesting parallels to the HCN channels. Thus, in CA1 pyramidal cells, Kv 4.2 (A-type) channels localize preferentially to the distal dendrites (Maletic-Savatic et al., 1995; Hoffman et al., 1997; Cai et al., 2004), where they control both backpropagation of action potentials into the dendritic tree (Hoffman et al., 1997; Bernard et al., 2004) and forward propagation of depolarization out of dendritic branches in which activated synapses are located (Cai et al., 2004; Chen et al., 2006). Like the HCN channels, the dendritic expression of Kv 4.2 channels commences early postnatally and then gradually increases with maturation (Maletic-Savatic et al., 1995; compare to Brewster et al., 2007a). Expression and subcellular trafficking of Kv 4.2 channels are regulated by neuronal activity (Tsaur et al., 1992; Bernard et al., 2004; Kim et al., 2007), and this regulation involves CaM kinase II (Varga et al., 2004), although it is not yet clear whether CaM kinase II also contributes to the transcriptional regulation of Kv 4.2, as it does for HCN1 (Richichi et al., 2008). In view of these parallels and the important integrative functions of both channel types in dendrites, one may suspect that they are homeostatically regulated: Indeed, data from invertebrate systems suggest that HCN- and A-type-channels are co-regulated on the transcriptional level (MacLean et al., 2003; Schulz et al., 2007). However, whether such co-regulation also occurs in mammalian neurons, and whether it includes developmental regulation, remains to be determined.

Homeostatic interaction may exist also between the HCN channels and others that contribute to the regulation of the resting membrane potential (e.g. Kir-, K_{leak} -, Na_{leak} - channels; Day et al., 2005; Meuth et al., 2006; Budde et al., 2008). However, information on the developmental regulation of these channels is sparse. In addition, mice deficient in the HCN1-isoform show no compensatory changes of the corresponding conductances, arguing against an extensive homeostatic linkage of these and HCN channels (Nolan et al., 2004, 2007). Further co-regulation of HCN- and other channels is apparent from the phenotype of mouse mutants such as *stargazer*, which is deficient in Ca^{2+} channel function (Letts et al., 1998), yet endowed with increased I_h in cortical neurons (DiPasquale et al., 1997 and see below).

5. Developmental dysregulation of HCN channels and brain pathology

Taken together, these findings support the framework of a coordinated expression of HCN channels in the developing hippocampus as an integral part of a developmental program that is regulated by neuronal activity. In this scenario, age-specific complements of HCN channel isoforms contribute to patterns of network activity. Network activity, in turn, promotes changes of HCN channel expression and distribution that support the integration of previously incoherent neuronal assemblies into a progressively maturing hippocampal network. This process requires a fine-tuned interplay between activity (i.e., the neuronal environment) and the HCN channels (i.e., determinants of intrinsic neuronal excitability) that is naturally sensitive to disturbances. Factors that interfere with this process, e.g., abnormal neuronal activity such as seizures, may have a long-lasting impact, because resulting errors (e.g. abnormal wiring) may be integrated into the developing network. In the worst case, these errors could promote pathological network function. Indeed, evidence for a critical role of a developmental dysregulation of HCN channels in the genesis of brain pathologies is emerging:

1) After experimental prolonged febrile seizures, hippocampal HCN channel expression and co-assembly were altered persistently. The coordinated expression changes were associated with a depolarization shift of h-channel activation and a slowing of the activation and deactivation kinetics of somatic and dendritic channels (Chen K. et al., 2001; Dyhrfjeld-Johnsen et al., 2008), thus modifying I_h and causing enduring hippocampal hyperexcitability

(Chen K. et al., 2001; Brewster et al., 2002, 2005; Santoro and Baram, 2003; Kamal et al., 2006; Dyhrfeld-Johnsen et al., 2008), that, in a subset of animals, may lead to frank epilepsy (Dube et al., 2006). In this context, it is worthwhile noting that abnormal expression levels of HCN1 channels have also been detected in the hippocampus of patients with temporal lobe epilepsy (associated with a history of early-life seizures; Bender et al., 2003). The full mechanistic relationship of the persistently altered HCN channel levels and the transformation of a 'normal' neuronal network to one generating spontaneous synchronized activity bursts (epileptic seizures) remains to be determined (Dube et al., 2007).

Recently, HCN expression and localization changes were also observed in adult models of temporal lobe epilepsy. Thus, down-regulation of HCN1 expression was reported after status epilepticus induced by kainic acid (Shah et al., 2004; Powell et al., 2008), pilocarpine (Jung et al., 2007) and after kindling (Powell et al., 2008). This down-regulation became specifically evident during the chronic period when epilepsy was established (Jung et al., 2007). These changes, although in some respects differing from the changes in the developing brain, generally support the notion of a potentially epileptogenic effect of seizure-induced HCN alterations. Importantly, several of these studies suggest that expression changes within the dendritic compartment could be particularly critical for epileptogenesis (Jung et al. 2007). In addition, reduced dendritic transport of HCN1 channels or reduced association of HCN1 with the adaptor protein TRIP8b in the chronic period (resulting in diminished transport of HCN1 channels to CA1 pyramidal cell dendrites) may also contribute to epileptogenesis (Shin et al., 2008). This new evidence supports a role for post-translational regulation of HCN subunit association in defining the distribution and function of these channels (Zha et al., 2008). In summary, HCN channels are altered by abnormal neuronal activity such as seizures in both mature and developing hippocampus, and these alterations accompany, and perhaps promote, the epileptogenic process. The precise role of these channels in governing neuronal excitability is still not fully understood, as discussed above, and may be age- and even model-specific (e.g., see Zhang et al., 2006).

2) Developmental dysregulation of cortical and/or thalamic HCN channel expression may contribute to epileptogenesis in genetic models of absence epilepsy: In two models, the Wistar Albino Glaxo/Rijswijk (WAG/Rij) and the Genetic Absence Epileptic Rats of Strasbourg (GAERS), the relative contribution of HCN1 channels to the channel pool and thalamic I_h was increased in thalamocortical relay (TC) neurons, resulting in reduced cAMP responsiveness of I_h (Budde et al., 2005; Kuisle et al., 2006). These changes were observed before the onset of absence seizures, suggesting that they might be a cause rather than a consequence of the seizure activity (Budde et al., 2005; Kuisle et al., 2006; Pape et al., 2007). Weakened cAMP actions could contribute to the generation of absences by facilitating burst discharges in TC neurons, promoting synchronization in the thalamocortical network (Budde et al., 2005; Kuisle et al., 2006). Similar mechanisms may underlie the spontaneous absence seizures in mice deficient in the HCN2 subunit (Ludwig et al., 2003). The basis of thalamic HCN1 upregulation in GAERS and WAG/Rij rats is unclear: Genomic analyses have not revealed mutations of the HCN1 gene (Gauguier et al., 2004; Rudolf et al., 2004) so that a developmental dysregulation due to abnormalities in the thalamic network (Dutuit et al., 2000, 2002) is plausible.

Dysfunction of cortical I_h might also contribute to corticothalamic network abnormalities, and the nature of the changes may be model-specific. Thus, in the *stargazer* mutant (see Section 4), enhanced I_h was observed in layer V pyramidal neurons of temporoparietal cortex, resulting in a lower threshold for aberrant thalamocortical spike-wave oscillations *in vivo* (DiPasquale et al., 1997). In contrast, in WAG/Rij rats with absence seizures, reduced I_h and lower expression levels of the HCN1 isoform compared to non-epileptic Wistar rats were found in pyramidal cells of somatosensory cortex (Strauss et al., 2004), and these changes were also associated with an increase in seizure activity (Schridde et al., 2006; Kole et al., 2007).

Interestingly, early suppression of seizure activity in WAG/Rij rats by chronic treatment with ethosuximide prevented the neocortical downregulation of HCN1 expression as well as the establishment of absence seizures, suggesting that in neocortex altered expression of HCN isoforms may be a consequence and not the cause of seizure activity (Blumenfeld et al., 2008). These findings further imply that seizures regulate HCN1 channel expression in cortex, as described above for hippocampus and thalamus.

It should be noted that the HCN channels are only one family of ion channels where developmental dysregulation may contribute to human disease and specifically to epilepsy: For example, the KCNQ2/3 channels generate a slowly-activating outward (potassium) current (I_M) upon membrane depolarization (Cooper and Yan, 2003). These channels are partially active at rest, and function to stabilize resting membrane potential and control repetitive neuronal firing. Mutations of these channels result in neonatal seizures (benign neonatal familial seizures, BNFC), and these seizures usually remit within the first months of life (Jentsch, 2000). Similarly, in rodents, controlled mutation of the channels during the 2–3 neonatal weeks, but not later in life, results in an epileptic phenotype (Peters et al., 2005). The basis of this age-specific pathology may derive from the fact that during the first weeks of life GABAergic inhibition is not yet fully established (Ben-Ari, 2002) so that KCNQ channels provide essential repolarizing, inhibitory function to the neuron (Okada et al., 2003). These functions may be supported by an age-specific expression of KCNQ isoforms or splice variants (Tinel et al., 1998; Smith et al., 2001; Weber et al., 2006; Geiger et al., 2006; Kanaumi et al., 2008). Later, when GABA assumes the role of the major inhibitor of neuronal firing, dysfunction of KCNQ2/3 channels no longer suffices to elicit abnormal hyperexcitability and seizures.

6. Conclusions and future perspectives

The roles of ion channels in neuronal networks are generally considered in the 3-dimensional mature network. This review introduces a fourth, developmental dimension, that consists of a dynamic and continuously changing vector. Using the HCN channels as examples, we find that the expression and the functions of the channels evolve with network development, and this evolution contributes to--and is in turn affected by--the developmental process. Disturbances of normal HCN channel function at a given developmental age will therefore lead to network derangements that differ from those caused by HCN channel dysfunction at a later age. Understanding the developmental aspects of ion channel expression, transport, assembly and function may thus provide us with valuable insights into the maturation of neuronal networks, as well as into the pathogenesis of certain developmental disorders.

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List of Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BNFC	benign neonatal familial seizures
fEPSP	field excitatory postsynaptic potential

GAERS

Genetic Absence Epileptic Rats of Strasbourg

GDP

giant depolarizing potential

HCN

hyperpolarization-activated cyclic nucleotide-gated channel

IPSC

inhibitory postsynaptic current

LTP

long-term potentiation

mPP

medial perforant path

NRSF/REST

neuronal restrictive silencing factor/RE1 silencing factor

STD

short-term depression

TC

thalamocortical

TTX

tetrodotoxin

WAG/Rij

Wistar Albino Glaxo/Rijswijk

References

- Agmon A, Wells JE. The role of the hyperpolarization-activated cationic current I(h) in the timing of interictal bursts in the neonatal hippocampus. *J Neurosci* 2003;23:3658–3668. [PubMed: 12736337]
- Altomare C, Terragni B, Brioschi C, Milanesi 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]
- Amaral, DG.; Witter, MP. The hippocampal formation. In: Paxinos, G., editor. *The rat nervous system*. 2. Academic Press; New York: 1995. p. 443-494.
- Antonucci DE, Lim ST, Vassanelli S, Trimmer JS. Dynamic localization and clustering of dendritic Kv2.1 voltage-dependent potassium channels in developing hippocampal neurons. *Neuroscience* 2001;108:69–81. [PubMed: 11738132]
- Aponte Y, Lien C, Reisinger E, Jonas P. Hyperpolarization-activated cation channels in fast-spiking interneurons of rat hippocampus. *J Physiol* 2006;574:229–243. [PubMed: 16690716]
- Araki T, Ito M, Oshima T. Potential changes produced by application of current steps in motoneurons. *Nature* 1961;191:1104–1105. [PubMed: 13683962]
- Bajarat R, Bräuer AU, Wasner U, Rölfs A, Strauss U. Functional significance of HCN2/3-mediated I(h) in striatal cells at early developmental stages. *J Neurosci Res* 2005;82:206–213. [PubMed: 16175581]
- Bayliss DA, Viana F, Bellingham MC, Berger AJ. Characteristics and postnatal development of a hyperpolarization-activated inward current in rat hypoglossal motoneurons in vitro. *J Neurophysiol* 1994;71:119–128. [PubMed: 7512625]
- Beaumont V, Zucker RS. Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic Ih channels. *Nat Neurosci* 2000;3:133–141. [PubMed: 10649568]

- Beck H, Yaari Y. Plasticity of intrinsic neuronal plasticity in CNS disorders. *Nat Rev Neurosci* 2008;9:357–369. [PubMed: 18425090]
- Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci* 2002;3:728–739. [PubMed: 12209121]
- Bender RA, Brewster AL, Santoro B, Ludwig A, Hofmann F, Biel M, Baram TZ. Differential and age-dependent expression of hyperpolarization-activated, cyclic nucleotide-gated cation channel isoforms 1–4 suggests evolving roles in the developing rat hippocampus. *Neuroscience* 2001;106:689–698. [PubMed: 11682156]
- Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci* 2003;23:6826–6836. [PubMed: 12890777]
- Bender RA, Galindo R, Mameli M, Gonzalez-Vega R, Valenzuela CF, Baram TZ. Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide gated (HCN) channel function. *Eur J Neurosci* 2005;2:2667–2674.
- Bender RA, Kirschstein T, Kretz O, Brewster AL, Richichi C, Rüschemschmidt C, Shigemoto R, Beck H, Frotscher M, Baram TZ. Localization of HCN1 channels to presynaptic compartments: novel plasticity that may contribute to hippocampal maturation. *J Neurosci* 2007;27:4697–4706. [PubMed: 17460082]
- Berger T, Larkum ME, Lüscher HR. High I(h) channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *J Neurophysiol* 2001;85:855–868. [PubMed: 11160518]
- Berger T, Senn W, Lüscher HR. Hyperpolarization-activated current Ih disconnects somatic and dendritic spike initiation zones in layer V pyramidal neurons. *J Neurophysiol* 2003;90:2428–2437. [PubMed: 12801902]
- Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* 2004;305:532–535. [PubMed: 15273397]
- Bernard C, Marcelin B, Esclapez M. Time- and spatial-dependent scaling of resonance with Ih properties in hippocampal pyramidal cells. *Soc Neurosci Abstr* 2007;37:587.11.
- Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, et al. Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia* 2008;49:400–409. [PubMed: 18070091]
- Brager DH, Johnston D. Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I(h) in hippocampal CA1 pyramidal neurons. *J Neurosci* 2007;27:13926–13937. [PubMed: 18094230]
- Bräuer AU, Savaskan NE, Kole MH, Plaschke M, Monteggia LM, Nestler EJ, Simburger E, Deisz RA, Ninnemann O, Nitsch R. Molecular and functional analysis of hyperpolarization-activated pacemaker channels in the hippocampus after entorhinal cortex lesions. *FASEB J* 2001;15:2689–2701. [PubMed: 11726545]
- Brewster AL, Bender RA, Chen Y, Dubé C, Eghbal-Ahmadi M, Baram TZ. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform and cell-specific manner. *J Neurosci* 2002;22:4591–4599. [PubMed: 12040066]
- Brewster AL, Bernard JA, Gall CM, Baram TZ. Formation of heteromeric hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the hippocampus is regulated by developmental seizures. *Neurobiol Dis* 2005;19:200–207. [PubMed: 15837575]
- Brewster AL, Chen Y, Bender RA, Yeh A, Shigemoto R, Baram TZ. Quantitative analysis and subcellular distribution of mRNA and protein expression of the hyperpolarization-activated cyclic nucleotide-gated channels throughout development in rat hippocampus. *Cereb Cortex* 2007a;17:702–712. [PubMed: 16648453]
- Brewster AL, Zha Q, Noam Y, Phan L, Baram TZ. Native hippocampal HCN channels: less promiscuous than in heterologous systems. *Soc Neurosci Abstr* 2007b;37:467.10.
- Brown HF, DiFrancesco D. Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J Physiol* 1980;108:331–351. [PubMed: 7230019]

- Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape HC. Impaired regulation of thalamic pacemaker channels through an imbalance of subunit expression in absence epilepsy. *J Neurosci* 2005;25:9871–9882. [PubMed: 16251434]
- Budde T, Coulon P, Pawlowski M, Meuth P, Kanyshkova T, Japes A, Meuth SG, Pape HC. Reciprocal modulation of I (h) and I (TASK) in thalamocortical relay neurons by halothane. *Pflügers Arch*. 2008;pub ahead of print
- Cai X, Liang CW, Muralidharan S, Kao JP, Tang CM, Thompson SM. Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* 2004;44:351–64. [PubMed: 15473972]
- Chan CS, Shigemoto R, Mercer JN, Surmeier DJ. HCN2 and HCN1 channels govern the regularity of autonomous pacemaking and synaptic resetting in globus pallidus neurons. *J Neurosci* 2004;24:9921–9932. [PubMed: 15525777]
- Chen K, Baram TZ, Soltesz I. Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. *Nat Med* 1999;5:888–894. [PubMed: 10426311]
- Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med* 2001;7:331–337. [PubMed: 11231632]
- Chen S, Wang J, Siegelbaum SA. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. *J Gen Physiol* 2001;117:491–504. [PubMed: 11331358]
- Chen X, Yuan LL, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D. Deletion of Kv4.2 gene eliminates dendritic A-type K⁺ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *J Neurosci* 2006;26:12143–12151. [PubMed: 17122039]
- Clapham DE. Not so funny anymore: Pacing channels are cloned. *Neuron* 1998;21:5–7. [PubMed: 9697846]
- Cobb SR, Larkman PM, Bulters DO, Oliver L, Gill CH, Davies CH. Activation of I_h is necessary for patterning of mGluR and mAChR induced network activity in the hippocampal CA3 region. *Neuropharmacology* 2003;44:293–303. [PubMed: 12604089]
- Constantine-Paton M, Cline HT. LTP and activity-dependent synaptogenesis: the more alike they are, the more different they become. *Curr Opin Neurobiol* 1998;8:139–148. [PubMed: 9568401]
- Cooper EC, Jan LY. M-channels: neurological diseases, neuromodulation and drug development. *Arch Neurol* 2003;60:496–500. [PubMed: 12707061]
- 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]
- Day M, Carr DB, Ulrich S, Ilijic E, Tkatch T, Surmeier DJ. Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2 and K_{leak} channels. *J Neurosci* 2005;25:8776–8787. [PubMed: 16177047]
- Dekker JP, Yellen G. Cooperative gating between single HCN pacemaker channels. *J Gen Physiol* 2006;128:561–567. [PubMed: 17043149]
- Denac H, Mevissen M, Scholtysik G. Structure, function and pharmacology of voltage-gated sodium channels. *Naumyn Schmiedeberg's Arch Pharmacol* 2000;362:453–479.
- Dickson CT, Magistretti J, Shalinsky MH, Fransen E, Hasselmo ME, Alonso A. Properties and role of I_h in the pacing of subthreshold oscillations in entorhinal cortex layer II neurons. *J Neurophysiol* 2000;83:2562–2579. [PubMed: 10805658]
- Dietrich D, Beck H, Kral T, Clusmann H, Elger CE, Schramm J. Metabotropic glutamate receptors modulate synaptic transmission in the perforant path: pharmacology and localization of two distinct receptors. *Brain Res* 1997;767:220–227. [PubMed: 9367251]
- DiFrancesco D, Tortora P. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature* 1991;351:145–147. [PubMed: 1709448]
- DiPasquale E, Keegan KD, Noebels JL. Increased excitability and inward rectification in layer V cortical pyramidal neurons in the epileptic mutant mouse Stargazer. *J Neurophysiol* 1997;77:621–631. [PubMed: 9065835]

- Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. *Ann Neurol* 2000;47:336–344. [PubMed: 10716253]
- Dube C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. *Brain* 2006;129:911–922. [PubMed: 16446281]
- Dube C, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. *Trends Neurosci* 2007;30:490–496. [PubMed: 17897728]
- Dutuit C, Didier-Bazes M, Vergnes M, Mutin M, Conjard A, Akoako A, Belin MF, Touret M. Specific alteration in the expression of glial fibrillary acidic protein, glutamate dehydrogenase, and glutamine synthetase in rats with genetic absence epilepsy. *Glia* 2000;32:15–24. [PubMed: 10975907]
- Dutuit M, Touret M, Szymocha R, Nehlig A, Behlin MF, Didier-Bazes M. Decreased expression of glutamate transporters in genetic absence epilepsy rats before seizure occurrence. *J Neurochem* 2002;80:1029–1038. [PubMed: 11953453]
- Dyhrfjeld-Johnsen J, Morgan RJ, Földy C, Soltesz I. Upregulated h-current in hyperexcitable CA1 dendrites after febrile seizures. *Front Cell Neurosci* 2008;2:pub ahead of print
- Falk T, Kilani RK, Strazdas LA, Borders RS, Steidl JV, Yool AJ, Sherman SJ. Developmental regulation of the A-type potassium-channel current in hippocampal neurons: role of the Kvbeta 1.1 subunit. *Neuroscience* 2003;120:387–404. [PubMed: 12890510]
- Fan Y, Fricker D, Brager DH, Chen X, Lu HC, Chitwood RA, Johnston D. Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). *Nat Neurosci* 2005;8:1542–1551. [PubMed: 16234810]
- Fain GL, Quandt FN, Bastian BL, Gerschenfeld HM. Contribution of a caesium-sensitive conductance increase to the rod photoresponse. *Nature* 1978;272:466–469. [PubMed: 416359]
- Fisahn A, Yamada M, Duttaroy A, Gan JW, Deng CX, McBain CJ, Wess J. Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. *Neuron* 2002;33:615–624. [PubMed: 11856534]
- Fogle KJ, Lyashchenko AK, Turbendian HK, Gibbs GR. HCN pacemaker channel activation is controlled by acidic lipids downstream of diacylglycerol kinase and phospholipase A2. *J Neurosci* 2007;27:2802–2814. [PubMed: 17360902]
- Fransen E, Alonso AA, Dickson CT, Magistretti J, Hasselmo ME. Ionic mechanisms in the generation of subthreshold oscillations and action potential clustering in entorhinal cortex layer II stellate neurons. *Hippocampus* 2004;14:368–384. [PubMed: 15132436]
- Franz O, Liss B, Neu A, Roeper J. Single-cell mRNA expression of HCN1 correlates with a fast gating phenotype of hyperpolarization-activated cyclic nucleotide-gated ion channels (Ih) in central neurons. *Eur J Neurosci* 2000;12:2685–2693. [PubMed: 10971612]
- Frere SG, Kuisle M, Lüthi A. Regulation of recombinant and native hyperpolarization-activated cation channels. *Mol Neurobiol* 2004;30:279–305. [PubMed: 15655253]
- Frotscher M, Drakew A, Heimrich B. Role of afferent innervation and neuronal activity in dendritic development and spine maturation of fascia dentata granule cells. *Cereb Cortex* 2000;10:946–951. [PubMed: 11007545]
- Garaschuk O, Hanse E, Konnerth A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J Physiol* 1998;507:219–236. [PubMed: 9490842]
- Gauguier D, van Luijckelaar G, Bihoreau MT, Wilder SP, Godfrey RF, Vossen J, Coenen A, Cox RD. Chromosomal mapping of genetic loci controlling absence epilepsy phenotypes in the WAG/Rij rat. *Epilepsia* 2004;45:908–915. [PubMed: 15270755]
- Gauss R, Seifert R, Kaupp UB. Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature* 1998;393:583–587. [PubMed: 9634235]
- Geiger J, Weber YG, Landwehrmeyer B, Sommer C, Lerche H. Immunohistochemical analysis of KCNQ3 potassium channels in mouse brain. *Neurosci Lett* 2006;400:101–104. [PubMed: 16513263]
- Goodman CS, Shatz CJ. Developmental mechanisms that generate precise pattern of neuronal connectivity. *Cell* 1993;72(Suppl):77–98. [PubMed: 8428376]
- Gravante B, Barbuti B, Milanese R, Zappi I, Viscomi C, DiFrancesco D. Interaction of the pacemaker channel HCN1 with filamin A. *J Biol Chem* 2004;279:43847–43853. [PubMed: 15292205]

- Grosse G, Draguhn A, Höhne L, Tapp R, Veh RW, Ahnert-Hilger G. Expression of Kv1 potassium channels in mouse hippocampal primary cultures: development and activity-dependent regulation. *J Neurosci* 2000;20:1869–1882. [PubMed: 10684888]
- Halliwel JV, Adams PR. Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res* 1982;250:71–92. [PubMed: 6128061]
- Harris EW, Cotman CW. Effects of synaptic antagonists on perforant path paired-pulse plasticity: differentiation of pre- and postsynaptic antagonism. *Brain Res* 1985;334:348–353. [PubMed: 2986785]
- Herrmann S, Stieber J, Ludwig A. Pathophysiology of HCN channels. *Pflugers Arch* 2007;454:517–22. [PubMed: 17549513]
- Hoffman DA, Magee J, Colbert CM, Johnston D. K⁺ channel regulation of signal propagation in dendrites of hippocampal pyramidal cell neurons. *Nature* 1997;387:869–875. [PubMed: 9202119]
- Hutcheon B, Yarom Y. Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends Neurosci* 2000;23:216–222. [PubMed: 10782127]
- Ishii TM, Takano M, Xie LH, Noma A, Ohmori H. Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. *J Biol Chem* 1999;274:12835–12839. [PubMed: 10212270]
- Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci* 2000;1:21–30. [PubMed: 11252765]
- Jung S, Jones TD, Lugo JN jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic channelopathy during epileptogenesis in the rat pilocarpin model of epilepsy. *J Neurosci* 2007;27:13012–13021. [PubMed: 18032674]
- Kamal A, Notenboom RG, de Graan PN, Ramakers GM. Persistent changes in action potential broadening and the slow afterhyperpolarization in rat CA1 pyramidal cells after febrile seizures. *Eur J Neurosci* 2006;23:2230–2234. [PubMed: 16630069]
- Kanaumi T, Takashima S, Iwasaki H, Itoh M, Mitsudome A, Hirose S. Developmental changes in KCNQ2 and KCNQ3 expression in human brain: possible contribution to the age-dependent etiology of benign familial neonatal convulsions. *Brain Dev* 2008;30:362–369. [PubMed: 18166285]
- Kaupp UB, Seifert R. Molecular diversity of pacemaker ion channels. *Annu Rev Physiol* 2001;63:235–257. [PubMed: 11181956]
- Kim J, Jung SC, Clemens AM, Petralia RS, Hoffman DA. Regulation of dendritic excitability by activity-dependent trafficking of the A-type K⁺ channel subunit Kv4.2 in hippocampal neurons. *Neuron* 2007;54:933–947. [PubMed: 17582333]
- Kimura K, Kitano J, Nakayama Y, Nakayishi S. Hyperpolarization-activated, cyclic nucleotide-gated HCN2 cation channel forms a protein assembly with multiple neuronal scaffold proteins in distinct modes of protein-protein interaction. *Genes Cells* 2004;9:631–640. [PubMed: 15265006]
- Klar M, Surges R, Feuerstein TJ. Ih channels as modulators of presynaptic terminal function: ZD7288 increases NMDA-evoked [3H]-noradrenaline release in rat neocortex slices. *Naunyn Schmiedeberg Arch Pharmacol* 2003;367:422–425. [PubMed: 12690435]
- Kole MH, Hallermann S, Stuart GJ. Single Ih channels in pyramidal neuron dendrites: properties, distribution, and impact on action potential output. *J Neurosci* 2006;26:1677–1687. [PubMed: 16467515]
- Kole MH, Bräuer AU, Stuart GJ. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol* 2007;578:507–525. [PubMed: 17095562]
- Kuisle M, Wanaverbecq N, Brewster AL, Frere SG, Pinault D, Baram TZ, Lüthi A. Functional stabilization of weakened thalamic pacemaker channel regulation in rat absence epilepsy. *J Physiol* 2006;575:83–100. [PubMed: 16728450]
- LeBlanc MO, Bland BH. Developmental aspects of hippocampal electrical activity and motor behavior in the rat. *Exp Neurol* 1979;66:220–237. [PubMed: 226389]
- Letts VA, Felix R, Biddlecome GH, Arikhat J, Mahaffey CL, Valenzuela A, Bartlett FS 2nd, Mori Y, Campbell KP, Frankel WN. The mouse stargazer gene encodes a neuronal Ca²⁺-channel gamma subunit. *Nat Genet* 1998;19:340–347. [PubMed: 9697694]

- Lörincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z. Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nat Neurosci* 2002;5:1185–1193. [PubMed: 12389030]
- 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]
- Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, et al. Absence epilepsy and dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J* 2003;22:216–224. [PubMed: 12514127]
- Lüthi A, McCormick DA. H-current: properties of a neuronal and network pacemaker. *Neuron* 1998;21:9–12. [PubMed: 9697847]
- Lujan R, Albasanz JL, Shigemoto R, Juiz JM. Preferential localization of the hyperpolarization-activated cyclic nucleotide-gated cation channel subunit HCN1 in basket cell terminals of the rat cerebellum. *Eur J Neurosci* 2005;21:2073–2082. [PubMed: 15869503]
- Lupica CR, Bell JA, Hoffman AF, Watson PL. Contribution of the hyperpolarization-activated current (I_h) to membrane potential and GABA release in hippocampal interneurons. *J Neurophysiol* 2001;86:261–268. [PubMed: 11431507]
- Maccaferri G, Manqoni M, Lazzari A, DiFrancesco D. Properties of hyperpolarization-activated current in rat hippocampal CA1 pyramidal cells. *J Neurophysiol* 1993;69:2129–2136. [PubMed: 7688802]
- Maccaferri G, McBain CJ. The hyperpolarization-activated current (I_h) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurons. *J Physiol* 1996;497:119–130. [PubMed: 8951716]
- MacLean JN, Zhang Y, Johnson BR, Harris-Warrick RM. Activity-independent homeostasis in rhythmically active neurons. *Neuron* 2003;37:109–120. [PubMed: 12526777]
- Magee JC. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J Neurosci* 1998;18:7613–7624. [PubMed: 9742133]
- Magee JC. Dendritic I_h normalizes temporal summation in hippocampal CA1 neurons. *Nat Neurosci* 1999;2:508–514. [PubMed: 10448214]
- Maletic-Savatic M, Lenn NJ, Trimmer JS. Differential spatiotemporal expression of K⁺ channel polypeptides in hippocampal neurons developing in situ and in vitro. *J Neurosci* 1995;15:3840–3851. [PubMed: 7751950]
- Mellor J, Nicoll RA, Schmitz D. Mediation of hippocampal mossy fiber long-term potentiation by presynaptic I_h channels. *Science* 2002;295:143–147. [PubMed: 11778053]
- Meuth SG, Kanyshkova T, Meuth P, Landgraf P, Munsch T, Ludwig A, Hofmann F, Pape HC, Budde T. Membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels. *J Neurophysiol* 2006;96:1517–1529. [PubMed: 16760342]
- Misonou H, Trimmer JS. Determinants of voltage-gated potassium channel surface expression and localization in Mammalian neurons. *Crit Rev Biochem Mol Biol* 2004;39:125–145. [PubMed: 15596548]
- Monteggia LM, Eisch AJ, Tang MD, Kaczmarek LK, Nestler EJ. Cloning and localization of the hyperpolarization-activated cyclic nucleotide-gated channel family in rat brain. *Mol Brain Res* 2000;81:129–139. [PubMed: 11000485]
- Moody WJ, Bosma MM. Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. *Physiol Rev* 2005;85:883–941. [PubMed: 15987798]
- Moosmang S, Biel M, Hofmann F, Ludwig A. Differential distribution of four hyperpolarization-activated cation channels in mouse brain. *Biol Chem* 1999;380:975–980. [PubMed: 10494850]
- Much B, Wahl-Schott C, Zong X, Schneider A, Baumann L, Moosmang S, Ludwig A, Biel M. Role of subunit heteromerization and N-linked glycosylation in the formation of functional hyperpolarization-activated cyclic nucleotide-gated channels. *J Biol Chem* 2003;278:43781–43786. [PubMed: 12928435]
- Narayanan R, Johnston D. Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. *Neuron* 2007;20:1061–75. [PubMed: 18093527]
- 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;115:551–164. [PubMed: 14651847]
- Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsaki G, Siegelbaum SA, Kandel ER, Morozov A. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell* 2004;119:719–732. [PubMed: 15550252]
- Nolan MF, Dudman JT, Dodson PD, Santoro B. HCN1 channels control resting and active integrative properties of stellate cells from layer II of the entorhinal cortex. *J Neurosci* 2007;27:12440–12451. [PubMed: 18003822]
- Noma A, Irisawa H. Membrane currents in the rabbit sinoatrial node cell as studied by the double microelectrode method. *Pflügers Arch* 1976;364:45–52.
- Notomi T, Shigemoto R. Immunohistochemical localization of Ih channel subunits, HCN1–4, in the rat brain. *J Comp Neurol* 2004;471:241–276. [PubMed: 14991560]
- Okada M, Zhu G, Hirose S, Ito K, Murakami T, Wakui M, Kaneko S. Age-dependent modulation of hippocampal excitability by KCNQ-channels. *Epilepsy Res* 2003;53:81–94. [PubMed: 12576170]
- Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Ann Rev Physiol* 1996;58:299–327. [PubMed: 8815797]
- Pape HC, Kanyshkova T, Broicher T, Budde T. Developmental and functional profile of the thalamic hyperpolarization-activated cation current, I_h , in absence epilepsy. *Thalamus & Related Systems*. 2007in press
- Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat Neurosci* 2005;8:51–60. [PubMed: 15608631]
- Pian P, Bucchi A, Robinson RB, Siegelbaum SA. Regulation of gating and rundown of HCN hyperpolarization-activated channels by exogenous and endogenous PIP2. *J Gen Physiol* 2006;128:593–604. [PubMed: 17074978]
- Pian P, Bucchi A, Decostanzo A, Robinson RB, Siegelbaum SA. Modulation of cyclic nucleotide-regulated HCN channels by PIP2 and receptors coupled to phospholipase C. *Pflügers Arch* 2007;455:125–145.
- Pike FG, Goddard RS, Suckling JM, Ganter P, Kasthuri N, Paulsen O. Distinct frequency preferences of different types of rat hippocampal neurones in response to oscillatory input currents. *J Physiol* 2000;529:205–213. [PubMed: 11080262]
- Poolos NP, Migliore M, Johnston D. Pharmacological up-regulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci* 2002;5:767–774. [PubMed: 12118259]
- Poolos NP, Bullis JP, Roth MK. Modulation of h-channels in hippocampal pyramidal neurons by p38 mitogen-activated protein kinase. *J Neurosci* 2006;26:7995–8003. [PubMed: 16870744]
- Powell KL, Ng C, O'Brien TJ, Xu SH, Williams DA, Foote SJ, Reid CA. Decreases in HCN mRNA expression in the hippocampus after kindling and status epilepticus in adult rats. *Epilepsia*. 2008epub ahead of print
- Proenza C, Tran N, Angoli D, Zahynacz K, Balcar P, Accili EA. Different roles for the cyclic nucleotide binding domain and amino terminus in assembly and expression of hyperpolarization-activated, cyclic nucleotide-gated channels. *J Biol Chem* 2002;277:29634–29642. [PubMed: 12034718]
- Qu J, Kryokova Y, Potapova IA, Doronin SV, Larsen M, Krishnamurthy G, Cohen IS, Robinson RB. MiRP1 modulates HCN2 channel expression and gating in cardiac myocytes. *J Biol Chem* 2004;279:43497–43502. [PubMed: 15292247]
- Richichi C, Brewster AL, Zha Q, Baram TZ. Activity-induced down-regulation of the hyperpolarization-activated cyclic nucleotide-gated (HCN) 1 channel expression is mediated by the Neuron Restrictive Silencing Factor NRSF/REST. *Soc Neurosci Abstr* 2007;37:467.11.
- Richichi C, Brewster AL, Bender RA, Simeone TA, Zha Q, Yin H, Weiss JH, Baram TZ. Mechanisms of seizure-induced 'transcriptional channelopathy' of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. *Neurobiol Dis* 2008;29:297–305. [PubMed: 17964174]
- Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol* 2003;65:453–480. [PubMed: 12471170]

- Rudolf G, Bihoreau MT, Godfrey RF, Wilder SP, Cox RD, Lathrop M, Marescaux C, Gauguier D. Polygenic control of idiopathic generalized epilepsy phenotypes in the genetic absence rats from Strasbourg (GAERS). *Epilepsia* 2004;45:301–308. [PubMed: 15030491]
- Santoro B, Baram TZ. The multiple personalities of h-channels. *Trends Neurosci* 2003;26:550–554. [PubMed: 14522148]
- Santoro B, Grant SGN, Bartsch D, Kandel ER. Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homology to Eag and cyclic nucleotide-gated channels. *Proc Natl Acad Sci USA* 1997;94:14815–14820. [PubMed: 9405696]
- 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]
- Santoro B, Chen S, Lüthi A, Pavlidis P, Shumyatsky GP, Tibbs GR, Siegelbaum SA. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J Neurosci* 2000;20:5264–5275. [PubMed: 10884310]
- Santoro B, Wainger BJ, Siegelbaum SA. Regulation of HCN channel surface expression by a novel C-terminal protein-protein interaction. *J Neurosci* 2004;24:10750–10762. [PubMed: 15564593]
- Schridde U, Strauss U, Bräuer AU, van Luijcklaar G. Environmental manipulations early in development alter seizure activity, Ih and HCN1 protein expression later in life. *Eur J Neurosci* 2006;23:3346–3358. [PubMed: 16820024]
- Schulz DJ, Goaillard JM, Marder EE. Quantitative expression profiling of identified neurons reveals cell-specific constraints on highly variable levels of gene expression. *Proc Natl Acad Sci U S A* 2007;104:13187–13191. [PubMed: 17652510]
- Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h-channels in entorhinal cortex layer III pyramidal neurons. *Neuron* 2004;44:495–508. [PubMed: 15504329]
- Shin M, Chetkovich DM. Activity-dependent regulation of h channel distribution in hippocampal CA1 pyramidal neurons. *J Biol Chem* 2007;282:33168–33180. [PubMed: 17848552]
- Shin M, Brager D, Jaramillo DC, Johnston D, Chetkovich DA. Mislocalization of h channel subunits underlies h channelopathy in temporal lobe epilepsy. *Neurobiol Dis*. 2008in press
- Simeone TA, Rho JM, Baram TZ. Single channel properties of hyperpolarization-activated cation currents in acutely dissociated rat hippocampal neurones. *J Physiol* 2005a;568:371–380. [PubMed: 16123099]
- Simeone TA, Dube C, Rho JM, Baram TZ. Properties of neuronal single-channel hyperpolarization-activated cation currents (I_H) are altered after experimental febrile seizures. *Soc Neurosci Abstr* 2005b;35:377.6.
- Sipilä ST, Huttu K, Soltesz I, Voipio J, Kaila K. Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. *J Neurosci* 2005;25:5280–5289. [PubMed: 15930375]
- Sipilä ST, Huutu K, Voipio J, Kaila K. Intrinsic bursting of immature CA3 pyramidal neurons and consequent giant depolarizing potentials are driven by a persistent Na^+ current and terminated by a slow Ca^{2+} -activated K^+ current. *Eur J Neurosci* 2006;23:2330–2338. [PubMed: 16706841]
- Smith JS, Jannotti CA, Dargis P, Christian EP, Aiyar J. Differential expression of *kenq2* splice variants: implications to m-current function during neuronal development. *J Neurosci* 2001;21:1096–1103. [PubMed: 11160379]
- Soleng AF, Chiu K, Raastad M. Unmyelinated axons in the rat hippocampus hyperpolarize and activate an H current when spike frequency exceeds 1 Hz. *J Physiol* 2003;552:459–470. [PubMed: 14561829]
- Southan AP, Morris NP, Stephens GJ, Robertson B. Hyperpolarization-activated currents in presynaptic terminals of mouse cerebellar basket cells. *J Physiol* 2000;526:91–97. [PubMed: 10878102]
- Strauss U, Kole MH, Bräuer AU, Pahnke J, Bajorat R, Rolfs A, Nitsch R, Deisz RA. An impaired neocortical Ih is associated with enhanced excitability and absence epilepsy. *Eur J Neurosci* 2004;19:3048–3058. [PubMed: 15182313]
- Stuart G, Spruston N. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *J Neurosci* 1998;18:3501–3510. [PubMed: 9570781]

- Surges R, Freiman TM, Feuerstein TJ. Input resistance is voltage dependent due to activation of I_h channels in rat CA1 pyramidal cells. *J Neurosci Res* 2004;76:475–480. [PubMed: 15114619]
- Surges R, Brewster AL, Bender RA, Beck H, Feuerstein TJ, Baram TZ. Regulated expression of HCN channels and cAMP levels shape the properties of the I_h -current in developing rat hippocampus. *Eur J Neurosci* 2006;24:94–104. [PubMed: 16882011]
- Tanaka S, Wu N, Hsiao CF, Turman J Jr, Chandler SH. Development of inward rectification and control of membrane excitability in mesencephalic v neurons. *J Neurophysiol* 2003;89:1288–1298. [PubMed: 12612052]
- Tansey EP, Chow A, Rudy B, McBain CJ. Developmental expression of potassium-channel subunit Kv3.2 within subpopulations of mouse hippocampal inhibitory interneurons. *Hippocampus* 2002;12:137–148. [PubMed: 12000114]
- Tinel N, Lauritzen I, Chouabe C, Lazdunski M, Borsotto M. The KCNQ2 potassium channel: splice variants, functional and developmental expression. Brain localization and comparison with KCNQ3. *FEBS Lett* 1998;438:171–176. [PubMed: 9827540]
- Tsaur ML, Sheng M, Lowenstein DH, Jan YN, Jan LY. Differential expression of K^+ channel mRNAs in the rat brain and down-regulation in the hippocampus following seizures. *Neuron* 1992;8:1055–1067. [PubMed: 1610565]
- Tsay D, Dudman JT, Siegelbaum SA. HCN1 channels constrain synaptically evoked Ca^{2+} spikes in distal dendrites of CA1 pyramidal neurons. *Neuron* 2007;56:1076–1089. [PubMed: 18093528]
- Ulen C, Tytgat J. Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem* 2001;276:6069–6072. [PubMed: 11133998]
- Ulrich D. Dendritic resonance in rat neocortical pyramidal cells. *J Neurophysiol* 2002;87:2753–2759. [PubMed: 12037177]
- van Welie I, van Hooft JA, Wadman WJ. Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated I_h channels. *Proc Natl Acad Sci U S A* 2004;101:5123–5128. [PubMed: 15051886]
- van Welie I, Remme MW, van Hooft JA, Wadman WJ. Different levels of I_h determine distinct temporal integration in bursting and regular-spiking neurons in rat subiculum. *J Physiol* 2006;576:203–214. [PubMed: 16809363]
- Varga AW, Yuan LL, Anderson AE, Schrader LA, Wu GY, Gatchel JR, Johnston D, Sweatt JD. Calcium-calmodulin-dependent kinase II modulates Kv4.2 channel expression and upregulates neuronal A-type potassium currents. *J Neurosci* 2004;24:3643–3654. [PubMed: 15071113]
- Vasilyev DV, Barish ME. Postnatal development of the hyperpolarization-activated excitatory current I_h in mouse hippocampal pyramidal neurons. *J Neurosci* 2002;22:8992–9004. [PubMed: 12388606]
- Vasilyev DV, Barish ME. Regulation of the hyperpolarization-activated cationic current I_h in mouse hippocampal pyramidal neurones by vitronectin, a component of extracellular matrix. *J Physiol* 2004;560:659–675. [PubMed: 15319414]
- Wang J, Chen S, Nolan MF, Siegelbaum SA. Activity-dependent regulation of HCN pacemaker channels by cyclic AMP: signaling through dynamic allosteric coupling. *Neuron* 2002;36:451–461. [PubMed: 12408847]
- Wang Z, Xu NL, Wu ZP, Duan S, Poo MM. Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* 2003;37:463–472. [PubMed: 12575953]
- Wang M, Ramos BP, Paspalas CD, Shu Y, Simen A, Duque A, Vijayraghavan S, Brennan A, Dudley A, Nou E, et al. Alpha2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell* 2007;129:397–410. [PubMed: 17448997]
- Weber YG, Geiger J, Kämpchen K, Landwehrmeyer B, Sommer C, Lerche C. Immunohistochemical analysis of KCNQ2 potassium channels in adult and developing mouse brain. *Brain Res* 2006;1077:1–6. [PubMed: 16500630]
- 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]
- Williams SR, Stuart GJ. Site independence of EPSP time course is mediated by dendritic $I(h)$ in neocortical pyramidal neurons. *J Neurophysiol* 2000;83:3177–3182. [PubMed: 10805715]

- Williams SR, Stuart GJ. Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *J Neurosci* 2003;23:7358–7367. [PubMed: 12917370]
- Ying SW, Jia F, Abbas SY, Hofmann F, Ludwig A, Goldstein PA. Dendritic HCN2 channels constrain glutamate-driven excitability in reticular thalamic neurons. *J Neurosci* 2007;27:8719–8732. [PubMed: 17687049]
- Zha Q, Brewster AL, Richichi C, Bender RA, Baram TZ. Activity-dependent heteromerization of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels: role of N-linked glycosylation. *J Neurochem* 2008;105:68–77. [PubMed: 17988239]
- Zhang W, Linden DJ. The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nat Neurosci Rev* 2003;4:885–900.
- Zhang K, Peng BW, Sanchez RM. Decreased Ih in hippocampal area CA1 pyramidal neurons after perinatal seizure-inducing hypoxia. *Epilepsia* 2006;47:1023–1028. [PubMed: 16822248]
- Zolles G, Klöcker N, Wenzel D, Weisser-Thomas J, Fleischmann BK, Roeper J, Fakler B. Pacemaking by HCN channels requires interaction with phosphoinositides. *Neuron* 2006;52:1027–1036. [PubMed: 17178405]
- Zong X, Eckert C, Yuan H, Wahl-Schott C, Abicht H, Fang L, Li R, Mistrik P, Gerstner A, Much B, et al. A novel mechanism of modulation of hyperpolarization-activated cyclic nucleotide-gated channels by Src kinase. *J Biol Chem* 2005;280:34224–34232. [PubMed: 16079136]

Figure 1. Molecular and functional characteristics of HCN channels and I_h

A) HCN channels are composed of four isoforms that can assemble as homomeric or heteromeric tetramers. Each of these isoforms, coded by the HCN1, 2, 3 and 4 genes, contains six transmembrane segments, with a positively charged S4 voltage sensor, similar to the voltage sensors of depolarization-activated potassium channels. HCN channels are non-selective cation channels that conduct primarily Na^+ ions at the negative membrane potentials at which they activate (Robinson and Siegelbaum, 2003). A characteristic feature of the HCN channels is the presence of a 120-amino-acid cyclic nucleotide binding domain in the cytoplasmic carboxy terminus (CNBD) which mediates their responses to cyclic AMP. B) Note: I_h activation following an action potential produces a slow depolarization that may activate other cation channels (Ca^{2+} , Na^+) and thus trigger a new action potential. I_h then deactivates (modified from Pape, 1996). C) Current clamp recordings illustrate the stabilizing actions of I_h on the resting membrane potential (dashed line): A hyperpolarizing input elicits a slow, depolarizing “sag” in membrane potential, reflecting I_h activation (red trace). Similarly, a depolarizing input yields a hyperpolarizing “sag” in membrane potential, reflecting I_h deactivation (blue trace). Note the rebound de- and hyperpolarization at the end of the hyperpolarizing resp. depolarizing input (arrows; modified from Poolos et al., 2002).

Figure 2. The subcellular localization of HCN channels in cortical and hippocampal neurons is neuron-type- and isoform-specific

HCN channels can localize to somatic, dendritic and axonal compartments of neurons, and these locations differ among neuronal populations. In CA1 pyramidal cells (A), HCN channels preferentially occupy dendritic locations (Lörincz et al., 2002; Notomi & Shigemoto, 2004; Brewster et al., 2007a), whereas in GABAergic interneurons, somatic HCN expression is more pronounced (B; Notomi & Shigemoto, 2004; Brewster et al., 2007a). Axonal localization is found in certain interneurons (e.g. basket cells, B; Notomi & Shigemoto, 2004; Aponte et al., 2006; Brewster et al., 2007a) and in layer II stellate cells of entorhinal cortex (C; Bender et al., 2007). The subcellular distribution is isoform-specific. Thus, HCN1- and HCN2-, but not HCN4-containing channels were detected in axon terminals of interneurons that express all three isoforms (B; Aponte et al., 2006; Brewster et al., 2007a).

Figure 4. Activity-dependent changes in HCN channel expression and molecular rearrangements
 Expression levels of HCN isoforms in developing hippocampus are differentially influenced by neuronal activity. Activity burst or seizures (red arrow in A) provoked reduction of the HCN1 isoform (fuchsia-colored spheres in A) and increased mRNA but not protein expression of the HCN2 isoform (yellow spheres in A). Decreased HCN1 protein levels further increased the stochastic probability of formation of HCN1/HCN2 heteromeric channels (A, green arrows), which may generate an h-current with distinctive biophysical properties (Chen S. et al., 2001). B) Seizure-induced expression changes of the HCN1 isoform (fuchsia-colored line) endured for months, suggesting that the normal developmental expression program of these channels (blue line) has been corrupted by the early-life seizures (red arrow).