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HCN and K_V7 (M-) channels as targets for epilepsy treatment

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Abstract

Voltage-gated ion channels are important determinants of cellular excitability. The Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) and K_V7 (M-) channels are voltage-gated ion channels. Both channels are activated at sub-threshold potentials and have biophysical properties that mirror each other. K_V7 channels inhibit neuronal excitability. Thus, mutations in K_V7 channels that are associated with Benign Familial Neonatal Convulsions (BFNC) are likely to be epileptogenic. Mutations in HCN channels have also been associated with idiopathic epilepsies such as GEFS+. In addition, HCN channel expression and function are modulated during symptomatic epilepsies such as temporal lobe epilepsy. It is, though, unclear as to whether the changes in HCN channel expression and function associated with the various forms of epilepsy promote epileptogenesis or are adaptive. In this review, we discuss this as well as the potential for K_V7 and HCN channels as drug targets for the treatment of epilepsy.

Voltage gated ion channels critically determine the intrinsic excitability of neurons as well as synaptic release. The distribution and properties of ion channels often differs between neurons in different brain regions (Nusser 2009). In addition, the location and biophysical properties of ion channels also varies within subcellular compartments (axons, dendrites and soma) of neurons. This variation in ion channel expression across cellular subtypes as well as within specific neuronal subcellular compartments is crucial for information processing and maintaining normal neural network excitability (Nusser 2009).

Alterations in voltage-gated ion channel expression and function have been associated with many different types of epilepsies (Steinlein and Noebels 2000; Lerche, Jurkat-Rott et al. 2001; Weber and Lerche 2008). Ion channels are also common targets for epilepsy treatment. Recent evidence suggests that the Hyperpolarization-activated Cyclic Nucleotide gated (HCN) and K_V7 (M- or KCNQ) channels are likely to be new exciting therapeutic targets for epilepsies. In this review, we will discuss the reasons for this.

1. Biophysical properties, subcellular localisation and function

HCN and K_V7 channels are subthreshold voltage-gated ion channels that activate and deactivate slowly with time constants in the order of 100 ms (Fig 1; (Biel, Wahl-Schott et al. 2009; Brown and Passmore 2009). HCN channels, as their name suggest, turn on at

potentials below -50 mV (Fig 1). In contrast, K_V7 channels, open at potentials more positive to -80 mV (Fig 1). Thus, both channels form a non-inactivating current at normal neuronal resting membrane potential (RMP). However, HCN channels are permeable to both Na^+ and K^+ and the current, I_h , is therefore, inward at rest and depolarizes the RMP (Pape 1996; Robinson and Siegelbaum 2003; Biel, Wahl-Schott et al. 2009; Shah, Hammond et al. 2010). The K_V7 or M- current, on the other hand, is outward at rest and hyperpolarizes the RMP (Shah, Migliore et al. 2008; Brown and Passmore 2009). Although HCN and K_V7 currents have counteractive effects on the RMP, they both reduce the membrane resistance (Robinson and Siegelbaum 2003; Hu, Vervaeke et al. 2007; Shah, Migliore et al. 2008; Biel, Wahl-Schott et al. 2009; Brown and Passmore 2009). Hence their effects on intrinsic excitability are complex.

4 HCN (HCN1-4) and 5 K_V7 ($K_V7.1$ - $K_V7.5$) channel subunits have been cloned. Both HCN and K_V7 channels exist as tetramers of their respective subunits and can form homomers. HCN channels are likely to exist as heteromers too (Biel, Wahl-Schott et al. 2009). In addition, $K_V7.3$ can form heteromers with $K_V7.2$ and $K_V7.5$ (Jentsch 2000; Delmas and Brown 2005; Brown and Passmore 2009). The gating and expression of K_V7 and HCN channels in neurons is significantly modulated by intracellular signalling molecules. HCN channels, as their name suggests, are affected by cyclic nucleotides such as cyclic adenosine 3',5' monophosphate (cAMP; (DiFrancesco and Tortora 1991; Wainger, DeGennaro et al. 2001; Wang, Chen et al. 2002; Wang, Ramos et al. 2007). The effect of cyclic nucleotides is subunit dependent with the activation curve of HCN2 and HCN4 being shifted much more to the right than that of HCN1 channels in heterologous systems (for an in depth review, see (Biel, Wahl-Schott et al. 2009)). Moreover, HCN and K_V7 channel activity is altered by phosphoinositides as well as kinases such as calcineurin and calcium/calmodulin dependent protein kinase II (for in depth reviews on the effects of signalling molecules on HCN channels and K_V7 channels see (Biel, Wahl-Schott et al. 2009) and (Delmas and Brown 2005) respectively).

HCN1, HCN2 $K_V7.2$, $K_V7.3$ and $K_V7.5$ expression is abundant in the hippocampus and cortex, areas of the brain known to be involved in idiopathic epilepsies as well as some types of acquired epilepsies (such as temporal lobe epilepsy; (Pape 1996; Jentsch 2000; Robinson and Siegelbaum 2003; Delmas and Brown 2005; Biel, Wahl-Schott et al. 2009; Brown and Passmore 2009). Interestingly, whilst the HCN subunits are highly localised to hippocampal and cortical pyramidal cell dendrites (Shah, Hammond et al. 2010), $K_V7.2$ and $K_V7.3$ are preferentially targeted to axons in these neurons (Devaux, Kleopa et al. 2004; Chung, Jan et al. 2006; Pan, Kao et al. 2006; Rasmussen, Frokjaer-Jensen et al. 2007; Shah, Migliore et al. 2008). This differential localisation in these neurons is important for understanding how these channels compute information. Consequently, whilst inhibition of HCN channels results in enhanced dendritic input resistance and lowering of RMP (Shah, Hammond et al. 2010), pharmacological block of K_V7 channels has little effect on dendritic input resistance (Hu, Vervaeke et al. 2007; Shah, Migliore et al. 2008; Shah, Migliore et al. 2011). Thus, HCN, but not K_V7 , channel inhibitors increase dendritic excitatory synaptic potential (EPSP) amplitude and prolong EPSP decay rate, thereby boosting EPSP summation (Fig 2; (Magee 1998; Stuart and Spruston 1998; Magee 1999; Magee 2000; Williams and Stuart 2000; Berger, Larkum et al. 2001; Poolos, Migliore et al. 2002; Shah, Anderson et al. 2004;

Huang, Walker et al. 2009; Shah, Hammond et al. 2010; Shah, Migliore et al. 2011). Hence, HCN channels exert a local effect on dendritic excitability. K_V7 , by being present at the axon initial segment, play a crucial role in regulating the action potential threshold (Shah, Migliore et al. 2008; Shah, Migliore et al. 2011). K_V7 channel inhibition results in a lower action potential threshold and a greater propensity for EPSP-spike coupling (Shah, Migliore et al. 2008; Shah, Migliore et al. 2011). As action potentials can back-propagate into dendrites, this may indirectly result in altered dendritic excitability (Shah, Migliore et al. 2008).

At the soma of cortical and hippocampal pyramidal neurons, though, where both channels are present, albeit at lower densities, treatment with either HCN or K_V7 channel inhibitors augments the input resistance, leading to enhanced EPSP amplitudes and slower decay. Consequentially, EPSP summation and EPSP-spike coupling is increased (Fig 2; (Magee 1998; Hu, Vervaeke et al. 2007; George, Abbott et al. 2009; Huang, Walker et al. 2009; Shah, Migliore et al. 2011). Since both HCN and K_V7 channels are voltage-dependent channels with different activation curves, the proportion of HCN and K_V7 channels open at a given potential is likely to vary. Hence, their combined effect on somatic EPSP kinetics and integration will depend on this factor (George, Abbott et al. 2009; Shah, Migliore et al. 2011). Therefore, their effects on somatic cell excitability are likely to be complex and reliant on whether both channels are modulated and whether the modulation results in up- or downregulation of their activity.

Besides pyramidal neuron dendrites, HCN and K_V7 channels are present in certain cortical synaptic terminals and interneurons too (Biel, Wahl-Schott et al. 2009; Brown and Passmore 2009). K_V7 channels have been postulated to reduce synaptic transmission and lower interneuron excitability (Martire, Castaldo et al. 2004; Lawrence, Saraga et al. 2006; Peretz, Sheinin et al. 2007; Luisi, Panza et al. 2009). On the other hand, depending on the other channels that are present, HCN channels can inhibit (Aponte, Lien et al. 2006) or enhance synaptic release (Bender, Soleymani et al. 2003; Huang, Lujan et al. 2011). HCN channels also have a varied effect on interneuron excitability. For example HCN channel blockers reduce the activity of CA1 oriens-lacunosum moleculare interneurons (Matt, Michalakis et al. 2011) but have little effect on the action potential firing frequency of fast-spiking interneurons in the dentate gyrus (Aponte, Lien et al. 2006). In contrast, a reduction in HCN channels following induction of temporal lobe epilepsy leads to an increase in the excitability of CA3 oriens-lacunosum moleculare interneurons (Dugladze, Vida et al. 2007). Hence, the overall effect on cortical and hippocampal neural network excitability caused by modulation of K_V7 and HCN channels is likely to depend on the change in the balance between excitation and inhibition.

In addition to the hippocampus and cortex, the thalamus also plays a critical role in certain epilepsies (e.g. absence epilepsy). All HCN subunits are expressed in the thalamus, though the cell-type localisation varies. Thus, HCN2, HCN4 and HCN1 are expressed in the adult thalamic dorsolateral geniculate nucleus and the thalamic ventrobasal complex whereas HCN3 is predominantly present in the intergeniculate leaflet (Roeloffs, Wickenden et al. 2008; Rogawski and Bazil 2008; Wickenden, Krajewski et al. 2008; Czuczwar, Wojtak et al. 2010). The thalamic relay neurons are the principle neurons in the thalamus (Pape 1996).

These are pacemaker neurons. The HCN current, I_h , together with the low threshold T-type Ca^{2+} channels is involved in generating this rhythmicity. I_h induces a slow depolarization that results in activation of T-type Ca^{2+} channels and action potentials (Pape 1996). This can enhance recurrent network activity in the thalamus. Indeed, I_h potentiation was found to enhance and accelerate oscillations within the thalamus (Yue and Huguenard 2001). Incomplete inhibition of I_h , on the other hand, led to slowing down of the oscillations and more robust thalamic responses, indicating that partial reduction in I_h may lead to epileptogenesis within the thalamus (Yue and Huguenard 2001).

HCN2 channels are present in reticular thalamic neurons too, which are GABAergic neurons (Luszczycki, Wu et al. 2009). Here, these are localized to dendritic spines and reduce excitatory synaptic summation in a similar manner to that in hippocampal and cortical pyramidal neurons (Luszczycki, Wu et al. 2009). Thus, inhibition of I_h led to enhanced inhibitory post-synaptic potential (IPSP) frequency in thalamic neurons (Luszczycki, Wu et al. 2009). Interestingly, these neurons also express K_v7/M -channels (Geiger, Weber et al. 2006). However, how these channels may affect neuronal firing or neural network excitability remains to be explored. Nevertheless, it is quite clear that there are a number of cells in different brain regions in which both HCN and K_v7 channels are present. In the next part of this review, we explore whether one or both channels are affected in various epilepsies and whether they can be useful targets for treatments.

2. K_v7 and HCN channel mutations associated with epilepsy

The underlying cause of epileptic seizures or syndromes can be symptomatic (including brain tumors, brain injury or stroke) or idiopathic (Steinlein and Noebels 2000; Lerche, Weber et al. 2005; Weber and Lerche 2008). Most idiopathic epilepsies are genetic in origin, with many of the epilepsy-associated genes encoding ion channels. Several mutations in K_v7/KCNQ genes have been associated with Benign Familial Neonatal Convulsion (BFNC), a syndrome characterized by the occurrence of seizures in the first few days of infancy and remitting within a few weeks (Biervert, Schroeder et al. 1998; Charlier, Singh et al. 1998; Singh, Charlier et al. 1998; Lerche, Biervert et al. 1999; Jentsch 2000; Coppola, Castaldo et al. 2003). These K_v7 mutations result in a decreased current when expressed in heterologous systems (Schroeder, Kubisch et al. 1998; Lerche, Biervert et al. 1999; Coppola, Castaldo et al. 2003). Interestingly, some of the mutations also disrupt K_v7 channel trafficking to axons in cultured hippocampal neurons (Chung, Jan et al. 2006). Altogether, these results suggest that these mutations are likely to enhance neuronal excitability by increasing EPSP-spike coupling and enhancing action potential firing frequency leading to augmented neurotransmitter release (Vervaeke, Gu et al. 2006; Yue and Yaari 2006; Hu, Vervaeke et al. 2007; Shah, Migliore et al. 2008). Consistent with this, mice that lack K_v7 subunit expression and mice in which mutated $\text{K}_v7.2$ subunits are knocked in, are epileptic (Peters, Hu et al. 2005; Otto, Yang et al. 2006; Singh, Otto et al. 2008). Unlike transgenic mice, though, the probability of recurrence of seizures in adulthood in humans is between 15-16% (Ronen, Rosales et al. 1993; Weber and Lerche 2008). It is unclear whether this discrepancy is due to species differences or other reasons such as the expression of different K_v7/KCNQ splice variants in the neonatal brain (Weber and Lerche 2008).

In contrast to K_v7 channels, relatively little is known about the association between HCN channel mutations in humans and idiopathic epilepsies. Mutations in HCN1 and HCN2 subunits have been discovered in patients with idiopathic generalised epilepsy (Tang, Sander et al. 2008). One such mutation, a recessive loss-of-function mutation in HCN2 that has been associated with idiopathic generalized epilepsy, has been shown to increase the excitability of rat cortical neurons (DiFrancesco, Barbuti et al. 2011). Interestingly, a gain of function mutation in HCN2 has been described in a small population of patients with febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+; (Dibbens, Reid et al. 2010). This is consistent with the increase in hippocampal HCN2 channel expression that has been reported in animal models of febrile seizures (Brewster, Bender et al. 2002). The significance of this for epileptogenesis, though, remains to be explored as mice lacking HCN2 channels have absence epilepsy (Ludwig, Budde et al. 2003). In addition, in a rat model of spontaneous absence epilepsy, cortical I_h is reduced, which may at least partially contribute to increased cortical neuron excitability (Strauss, Kole et al. 2004; Kole, Brauer et al. 2007). I_h in thalamic relay neurons, though, is unaltered in pre-epileptic WAG/Rij or GAERS rats, which are recognized genetic models of absence epilepsy (Budde, Caputi et al. 2005; Kuisle, Wanaverbecq et al. 2006). There is, though, altered HCN isoform expression such that HCN1 expression is enhanced in the thalamus of these rodents (Budde, Caputi et al. 2005; Kuisle, Wanaverbecq et al. 2006; Brodie 2010). As HCN1 is less sensitive to cAMP compared with HCN2 and HCN4, the sensitivity of I_h to cAMP is reduced in thalamic neurons in the WAG/Rij and GAERS rats, which may facilitate burst discharges in thalamic neurons, leading to enhanced spontaneous synchronization of thalamic and reticular neurons (Budde, Caputi et al. 2005; Kuisle, Wanaverbecq et al. 2006). It remains to be determined if this may lead to spike-wave-discharges that are characteristic of absence epilepsy.

3. Seizure-induced alterations in HCN/ K_v7 channel function

There is considerable evidence in animal models and humans to suggest that HCN channel function is altered as a consequence of seizure-activity. This is perhaps not surprising as HCN channels are highly susceptible to plasticity (Biel, Wahl-Schott et al. 2009). Seizure-induced plasticity of HCN channels was first detected in a model of febrile seizures (Chen, Wang et al. 2001). I_h was found to be augmented both at the soma and dendrites of hippocampal CA1 pyramidal neurons obtained from adults that had experienced febrile seizures several weeks prior (Chen, Wang et al. 2001; Dyhrfjeld-Johnsen, Morgan et al. 2008). In addition, there was a reduction in hippocampal HCN1 subunit expression and an increase in HCN2 subunits (Brewster, Bender et al. 2002). Interestingly, a decrease in dendritic K^+ channel function was detected too (Dyhrfjeld-Johnsen, Morgan et al. 2008). Hence, though the cells were hyperexcitable, the underlying mechanism(s) responsible for this are unclear. Further, whether I_h is epileptogenic under these conditions remains to be evaluated as only a third of the rodents subjected to febrile seizures develop chronic epilepsy (Walker and Kullmann 1999; Dube, Brewster et al. 2009).

Seizure-induced alterations in I_h and HCN protein levels have also been found in animal models of temporal lobe epilepsy (TLE) as well as cortical tissue obtained from TLE patients. TLE is a common form of adult epilepsy, which is initiated by a brain insult or

trauma (e.g. status epilepticus or brain tumors; (Engel 1996). A characteristic feature of this disorder is a seizure-free period, the so-called latent period, between the precipitating factor and the onset of chronic epilepsy (spontaneous behavioural seizures; (Engel 1996). Many of the clinical symptoms of this disorder can be mimicked in rodents by the administration of chemoconvulsants such as kainic acid (White 2002). Using the kainic acid model, it was shown that I_h and HCN protein levels were reduced in the entorhinal cortex (EC) within 24 hr of the first seizure (Shah, Anderson et al. 2004). This decrease in I_h was persistent for at least one week. In addition, EC layer III pyramidal soma and dendrites were hyperexcitable. The dendritic hyperexcitability, in particular, could be mimicked in wildtypes using the I_h inhibitor, ZD7288, suggesting that the reduction in I_h could at least partly contribute to this. Similar results have been found in hippocampal CA1 pyramids and O-LM interneurons in animal models at the acute and chronic stages of the condition (Dugladze, Vida et al. 2007; Jung, Jones et al. 2007; Shin, Brager et al. 2008; Marcelin, Chauviere et al. 2009; Jung, Warner et al. 2011). In addition, a loss in I_h function has been found in cortical neurons present in chronically epileptic tissue obtained from patients (Wierschke, Lehmann et al. 2010). The changes in HCN protein levels observed in all animal models as well as humans are likely to be due to transcriptional as well as post-translational changes (Bender, Soleymani et al. 2003; Powell, Ng et al. 2008; Jung, Bullis et al. 2010; McClelland, Flynn et al. 2011). Further, seizure induced changes in activity-dependent trafficking may also be a contributing factor (Shin, Brager et al. 2008).

Although a reduction in I_h enhances pyramidal cell activity, the effects on neural network excitability may be more complex as HCN channels are expressed post-synaptically in interneurons as well as pre-synaptically in interneurons and principle neurons (Biel, Wahl-Schott et al. 2009). It is not known if seizure activity affects HCN channel expression in all cell types and neuronal subcellular compartments in a similar manner. Therefore, does a reduction in I_h following the induction of TLE facilitate the process of epileptogenesis? HCN1 null mice have been used to address this question as HCN2 null mice have absence epilepsy (Ludwig, Budde et al. 2003). HCN1 null mice do not have spontaneous seizures and the EEG also appears to be normal (Nolan, Malleret et al. 2003; Nolan, Malleret et al. 2004; Huang, Walker et al. 2009; Santoro, Lee et al. 2010). The mice though were more susceptible to chemoconvulsants such as kainic acid and pilocarpine as well as kindling (Fig 3; (Huang, Walker et al. 2009; Santoro, Lee et al. 2010). In addition, the latent period was significantly shorter (Fig 3; (Huang, Walker et al. 2009). Moreover, although the EEG appeared to be similar in wildtypes and HCN1 null mice, the balance between excitatory and inhibitory inputs was altered in the entorhinal cortex such that the frequency of excitatory inputs far exceeded the inhibitory inputs in HCN1 null tissue whereas the converse occurred in wildtype tissue (Huang, Walker et al. 2009). It is likely that this is not the case in all parts of the cortex or in other brain regions. Indeed, in the neocortex, tonic GABA currents have been shown to be upregulated in HCN1 null mice (Chen, Shu et al. 2010). This might thus explain why these mice are not epileptic. Nonetheless, there is substantial evidence to suggest that a decrease in HCN channel function may aid the process of epileptogenesis in some forms of epilepsy.

In contrast to HCN channels, there is very little evidence that K_v7 channel expression and function are altered by seizure activity. Interestingly, in human tissue obtained from TLE

patients, $K_V7.5$ expression appeared to be not dissimilar to control tissue (Zolles, Wenzel et al. 2009). It is, though, possible that the function of the channels is impaired. Nevertheless, this indicates that K_V7 channel modulators may be effective for treatment of patients with TLE. Indeed, the K_V7 channel enhancers, retigabine and ICA-27243, have been shown to effectively suppress seizures in TLE models (Rostock, Tober et al. 1996; Tober, Rostock et al. 1996; DiFrancesco, Barbuti et al. 2011).

4. Therapeutic potential for HCN and K_V7 channel modulators

Consistent with the role of K_V7 channels in dampening neuronal excitability, the K_V7 channel enhancer, retigabine (ezogabine) is effective as an anticonvulsant in several animal models of epilepsy (Rogawski and Bazil 2008; Czuczwar, Wojtak et al. 2010). Phase III clinical trials showed that retigabine suppressed between 45-50% of baseline seizures in patients with partial onset seizures (Czuczwar, Wojtak et al. 2010). Therefore, it has recently received approval in Europe and USA as an adjunct for the treatment of partial epilepsy. Retigabine, however, at high concentrations can also act as a $GABA_A$ receptor agonist (Rogawski and Bazil 2008; Czuczwar, Wojtak et al. 2010). Its effects though are likely to be via enhancing K_V7 channel activity as another more selective K_V7 channel enhancer, ICA-27243 is highly effective as a broad-spectrum anticonvulsant in animal models of epilepsy (Roeloffs, Wickenden et al. 2008).

In contrast to K_V7 channels, the role of HCN channels in epilepsy is clearly more complex. In some forms of epilepsy such as TLE, enhancing HCN channel function may be beneficial. Some anticonvulsants such as lamotrigine and gabapentin have been suggested to increase HCN channel activity at rest (Poolos, Migliore et al. 2002; Surges, Freiman et al. 2003). However, these compounds do have other modes of action (Brodie 2010) and it is unclear if their effects on seizure activity are due to enhancing HCN channel function or due to their other effects. Can increasing HCN channel activity alter epileptogenesis? It has recently been demonstrated that increasing HCN channel expression by disrupting the interaction between the Neuron-Restrictive Silence Factor (NRSF) and HCN1 restores HCN channel function in CA1 pyramidal dendrites 3 days following status epilepticus and thereby delays the onset of spontaneous seizure activity (McClelland, Flynn et al. 2011). This did not, though, prevent spontaneous seizure activity as a number of other mechanisms such as altered phosphorylation signalling may contribute to the persistent downregulation of HCN channel function too. Certainly a recent study has shown that in chronically epileptic animals, calcineurin and p38 MAPK activity is enhanced and reduced respectively. Consequently, the activation curve of I_h in chronically 'epileptic' hippocampal CA1 neurons is shifted to the left, resulting in fewer HCN channels being available at rest (Jung, Bullis et al. 2010). Since HCN channel activity is altered in a significant number of different cell types following the induction of epilepsy, it is unclear whether these phosphorylation dependent changes occur in all of these cell types. An alternate approach may be to better understand the cellular mechanisms by which a reduction in I_h leads to enhanced epilepsy. Would targeting these be more beneficial for the treatment of certain forms of epilepsy? In addition, for other forms of epilepsy (e.g. febrile seizures) would inhibiting I_h suppress seizure activity? Undoubtedly, further work is required to evaluate the link between I_h and epilepsy.

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A K_V7/M - channel biophysical properties in hippocampal neurons

(i) K_V7 channel characteristics measured using a classical de-activation protocol

(ii) Activation curve

B HCN channel biophysical properties in cortical neurons

(i) HCN channel activation characteristics

(ii) Activation curve

Fig 1. HCN and K_V7/M - channel biophysical properties

A(i) Example recordings of M-current de-activation in hippocampal pyramidal in response to hyperpolarizing potentials from a holding potential of -20 mV. Since there are a number of other conductances also present in these neurons, the recordings were obtained in the absence and presence of the K_V7/M - channel inhibitor, XE991 ($3 \mu\text{M}$). The 'difference' current represents the M-current de-activation. (ii) The apparent activation curve of K_V7 current produced from experimentally obtained conduction values as detailed in Shah et al. (2008). B(i) Example trace of the HCN channel current recorded from an entorhinal cortical dendrite when it was hyperpolarized to -150 mV from a holding potential of -40 mV. The recording was made in a cell-attached mode. B(ii) The activation curve of I_h obtained using cell-attached recordings made from entorhinal cortical neuron dendrites. A(i) and A(ii) have been adapted from Shah et al. (2008) whereas B(i) and B(ii) have been adapted from Shah et al. (2004).

Fig 2. Modulation of EPSP integration by HCN and K_v7/M- channel inhibitors

A Example recordings showing the effects of the HCN channel inhibitor, ZD7288 (ZD, 15 μ M) and the K_V7/M -channel blocker, XE991 (3 μ M) on entorhinal cortical (EC) dendritic α EPSP summation and hippocampal dendritic α EPSP integration respectively. α EPSPs were obtained by current injection of the alpha waveform (for methods see (Shah, Anderson et al. 2004)). B Traces demonstrating the effects of ZD7288 and XE991 on α EPSP summation in EC and hippocampal soma respectively. The figure has been adapted from (Shah, Anderson et al. 2004) and (Shah, Migliore et al. 2011).

A Enhanced susceptibility of HCN1^{-/-} mice to kainic acid induced seizures

(i) Wildtype + 10 mg/kg kainic acid

(ii) HCN1^{-/-} + 10 mg/kg kainic acid

(iii) Wildtype + 20 mg/kg kainic acid

B Significantly shorter latent period duration in HCN1^{-/-} mice

(i) EEG from HCN1^{-/-} mouse 2 days following SE termination

(ii) EEG from wildtype mouse 2 days following SE termination

Fig 3. Seizure susceptibility of HCN1 null mice

A(i), A(ii) and A(iii) Example traces showing the electroencephalography (EEG) recorded from HCN1 null mice and wildtype littermates when administered kainic acid either at concentrations of 10 mg/kg or 20 mg/kg. A(iv) Graph summarising the time taken to induce status epilepticus (SE) with the varying doses of kainic acid. NB Administration of 20 mg/kg kainic acid to HCN1 null mice was lethal whilst treatment with 10 mg/kg kainic acid had little effect on wildtypes for up to 4 hr. B(i) and B(ii) EEG recordings from HCN1 null and wildtype mice 2 days following termination of SE induced with 10 mg/kg and 20 mg/kg

kainic acid respectively. The arrows in B(ii) mark the presence of interictal spikes B(iii) Graph showing the latent period duration (i.e. the time between termination of SE and detection of the first spontaneous seizure) in HCN1 null and wildtype mice when treated with kainic acid. The figure has been adapted from (Huang, Walker et al. 2009).