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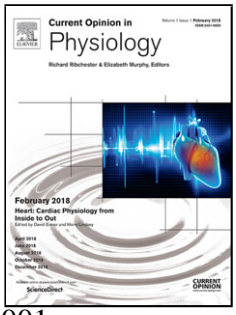

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Neuronal HCN channel function and plasticity

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Short title: HCN channel function

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Abstract:

The hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channel is a voltage-gated cation channel that is activated with hyperpolarization. Four subunits, HCN1 -4, have thus far been identified. All four subunits are expressed in the central nervous system (CNS), though their expression pattern varies considerably. In many CNS neurons, HCN channels are localised to somato-dendritic compartments where they regulate the resting membrane potential and membrane resistance, and thereby affect synaptic potential shapes and integration and neuronal firing patterns. Emerging evidence suggests that HCN channels are also present within certain axons and synaptic terminals. Modulation of presynaptic HCN channel activity leads to altered synaptic release in a synapse-specific manner. Given that HCN channel function can be modified by activity-dependent and neurotransmitter receptor activation, HCN channels may diversely affect neuronal and network excitability, thereby affecting physiological states such as learning and memory as well as pathophysiological conditions such as epilepsy and depression.

Highlights:

1) Postsynaptic HCN channels affect neuronal intrinsic membrane properties

2) Postsynaptic HCN channels limit synaptic potential decay, summation and plasticity

3) HCN channels are located presynaptically too in select axons and synaptic terminals

4) Presynaptic HCN channels modify synaptic release and neuronal excitability

5) I_h influences physiological states such as learning and disorders such as epilepsy.

Voltage-gated ion channels play a critical role in regulating neuronal intrinsic and synaptic excitability. The hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels are voltage-gated ion channels that open at potentials more negative to -50 mV and are thus, active at the normal neuronal resting membrane potential (RMP). The channels are also permeable to both Na^+ and K^+ ions, forming a depolarizing current at rest that contributes to influencing neuronal activity. Further, these channels influence the membrane resistance and thereby affect post-synaptic potential shapes and integration (Fig 1). Hence, HCN channels play a crucial role in maintaining neuronal activity [1-3].

Thus far, four HCN subunits, HCN1-4, have been cloned [4, 5]. These subunits are diversely located throughout the central nervous system (CNS), with HCN1 subunits being predominantly present in the cortex, hippocampus, cerebellum and brain stem. HCN2 subunits, on the other hand, are mainly situated in areas such as the thalamus and brain stem. HCN3 subunits are expressed at low levels in the CNS whilst HCN4 subunits are highly localised to specific regions such as the olfactory bulb [6, 7]. These HCN subunits can form homomeric or heteromeric channels when expressed in heterologous systems. Here, the HCN1-4 homomeric channels have distinct activation time constants. HCN1 homomeric channels have a fast activation time constant whilst HCN4 homomeric channels have a very slow activation time constant. HCN2 and HCN3 channels have intermediate activation time constants [4, 5]. Further, though cyclic nucleotides modify their activity, the extent to which the homomeric HCN channels are regulated varies considerably. Moreover, other intracellular signalling molecules such as phosphoinositides and kinases as well as auxiliary subunits such as TPR-containing Rab8b interacting protein (TRIP8b) modify the biophysical properties and expression profile of HCN subunits in heterologous systems as well as neurons [1-3]. The differential modulation of HCN subunits by various intracellular molecules as well as their distinct intrinsic biophysical characteristics will contribute to their diverse effects on neuronal excitability. In this review, I will discuss the various mechanisms by which HCN channels affect neuronal function in the CNS.

Post-synaptic HCN channels and neuronal excitability

HCN1 channels are located in the somata of many principal neurons and a subset of interneurons within the CNS [8-13]. Here, whilst inhibition of these channels augments the membrane resistance, the resultant hyperpolarization means that there is little effect on action potential firing induced by depolarization potentials [8-10, 12, 13]. Interestingly, in cerebellar purkinje neurons, by affecting the neuronal RMP, I_h may influence the switch between tonic firing and quiescent states *in vivo* [13, 14]. The spontaneous firing patterns of purkinje cells have a significant impact on other neurons in the deep cerebellar nuclei and may play a significant role in motor learning. Indeed, deletion of HCN1 subunits, which encodes I_h in these neurons, impairs learning of certain motor behaviours [15].

Intriguingly, immunohistochemical and electrophysiological studies showed that HCN channels are located in high densities within pyramidal neuron dendrites in the hippocampus and cortex [7, 12, 16-20]. Here, by significantly reducing the membrane resistance, the HCN channel current, I_h, limits low threshold Ca^{2+} channel activity and thereby, excitatory postsynaptic potential (EPSP) amplitudes and decay as well as the occurrence of dendritic Ca^{2+} spikes caused by depolarizing potentials [21]. Thus, I_h restricts summation of trains of EPSPs and dendritic excitability in these neurons (Fig 1). Hence, inhibition of I_h boosts dendritic EPSP summation and EPSP-spike coupling, despite hyperpolarization of the RMP [12, 17, 22]. By augmenting EPSP summation, a decrease in I_h also increases the propensity for long-term potentiation (LTP) to occur within these neurons [23-27]**. Indeed, LTP and

spatial memory is greater in HCN1 null mice in which I_h in hippocampal pyramidal cell dendrites is reduced significantly compared with wildtypes [25]. Further, hippocampal place cell and entorhinal grid cell firing rate maps are larger in HCN1 null mice than in wildtypes [28, 29]. Hence, I_h maintains neuronal and network activity. Certainly, HCN1 null mice are more susceptible to chemoconvulsant or kindling-induced seizures and epilepsy [22, 30].

In addition to changes in EPSP summation, I_h also affects the shapes and integration of somato-dendritic inhibitory post-synaptic potentials (IPSPs). As I_h is activated by hyperpolarization, the current will be further activated during trains of IPSPs. As I_h is a depolarizing current, this will limit further synaptic hyperpolarization [31-33]. In cortical neurons, distal dendritic I_h, influenced local dendritic IPSP amplitude and timecourse substantially [33]. In contrast, due to the lower density, somatic I_h had little effect on somatic IPSPs. Nonetheless, the significant effects of distal I_h on IPSPs restricted axo-somatic depolarization [33]. Interestingly, in these neurons, the activation of I_h overlaps with that of the persistent Na^+ current (I_{NaP}), such that inhibition of I_h led to IPSP amplification by I_{NaP}. This effect, though, appears to be cell-type dependent because in subthalamic neurons, the activation of HCN channels during synaptic inhibition constrained de-inactivation of T-type Ca^{2+} channels and rebound action potential firing [31]. Thus, the effects of I_h on synaptic integration are likely to be vary depending on the other ion channels located nearby.

Somato-dendritic I_h also plays a key role in the generation and modulation of intrinsic and network oscillations [34-38]. In entorhinal cortical layer II stellate neurons and thalamocortical relay neurons, I_h together with other subthreshold-active currents such as the I_{NaP} generates intrinsic oscillations that modulate neuronal activity [34, 35]. Interestingly, intrinsic oscillations in thalamic neurons are enhanced in HCN2 null mice, promoting low threshold bursting and associated spike-wave discharges underlying absence epilepsy [37]. Further, changes in I_h affect network oscillations, though the underlying cellular mechanisms for this phenomenon are unclear [25, 28]. Hence, I_h modulates neural intrinsic and network activity in diverse ways.

Post-synaptic HCN channel modulation and plasticity

As HCN channel function is significantly affected by intracellular signalling molecules, changes in the activity of these induced by altered intrinsic neuronal or receptor function may result in variations in neuronal I_h. Consequently, neuronal excitability and behaviour will be altered. Indeed, variations in Ca^{2+} influx caused by enhanced synaptic input or intrinsic neuronal firing results in activation of various intracellular molecules such as CaMKIIα. This in turn gives rise to altered I_h amplitude or kinetics and homeostatic adjustment of neuronal excitability [23, 24, 26, 27, 38, 39]. Certainly, receptor-dependent reduction in I_h amplitude affects the induction of LTP in hippocampal and prefrontal pyramidal neurons, bringing about enhanced working and spatial memory **[27, 40]. Further, modulation of I_h by intracellular molecules is likely to be a robust mechanism for altering the periodicity of intrinsic neuronal oscillations [11, 41].

Interestingly, in olfactory bulb mitral cells, synaptic input affects expression of HCN channels within individual glomeruli. As I_h affects post-synaptic potential processing, mitral cells in different glomeruli with distinct odour receptors will process odour-related information differentially [42]*. Activity-dependent modulation of HCN channels is also likely to be a critical mechanism for the dysfunction of these channels in principal neurons of the anterior cingulate cortex following sciatic nerve injury [43]**. In this case, activation of 5-hydroxytryptamine (5-HT) receptors rescues the activity-dependent modification in HCN channel activity, normalises the response of the neurons and alleviated the associated

mechanical pain hypersensitivity in nerve-injured animals [43]**. Thus, receptor-dependent alterations in HCN channel activity may be a potential, new mechanism for the treatment of some neuronal disorders.

In addition to modulation by intracellular molecules such as kinases, HCN channels are actively trafficked in post-synaptic compartments by binding to chaperone proteins known as TPR-containing Rab8b interacting protein (TRIP8b) [44-46]. TRIP8b also shifts the I_h activation curve to the left. Moreover, the presence of TRIP8b alters sensitivity of HCN channels to cyclic nucleotides [46]. Intriguingly, the absence of TRIP8b not only alters post-synaptic HCN channel expression and function, it also leads to altered synaptic plasticity in hippocampal neurons [47]. Further, by modification of integration of synaptic inputs, the lack of TRIP8b and HCN channels in post-synaptic compartments may contribute to pathological disorders such as depression and anxiety [48]. Moreover, given that TRIP8b regulates expression of both HCN1 and HCN2 subunits, TRIP8b null mice also have absence epilepsy [48]. As yet, it is unknown if TRIP8b expression and function is altered by intracellular molecules. Nonetheless, activity- and receptor- dependent modifications in I_h *in vivo* are likely to be a significant mechanism for altering neuronal excitability, plasticity and physiological states such as learning and memory.

Pre-synaptic HCN channel function and plasticity

Intriguingly, there is increasing evidence that HCN1 channels are located in axons and presynaptic terminals where they regulate action potential firing and synaptic release. Presynaptic I_h was initially shown to modulate synaptic strength and promote long-lasting facilitation at the crayfish neuromuscular junction [49, 50]. In mammals, I_h was also identified in cerebellar basket cell synapses, where it modified inhibitory synaptic release [51], and in excitatory synaptic terminals of the auditory brainstem [52]. There has been considerable immunohistochemical evidence since, showing that HCN subunits may be concentrated in synaptic terminals in a wide variety of brain regions including the retina [53], cerebellum [54], globus pallidus [55] and hippocampus [7]. Interestingly, HCN channel subunits were predominantly located in inhibitory synaptic terminals in these regions [7]. The cellular mechanisms by which presynaptic I_h may alter synaptic transmission from inhibitory synapses is still unclear [51, 55, 56].

HCN channels are also located in selective excitatory synaptic terminals throughout the CNS. In the medial entorhinal cortex (mEC), HCN1 channels restrict glutamatergic release from a subset of adult excitatory synaptic terminals predominantly targeted to layer III (LIII) pyramidal neurons by limiting Ca^{2+} influx via T-type Ca^{2+} channels [57]** (Fig 2). Further, two-photon imaging showed that FM1-43 dye release from a subset of terminals within mEC LIII was enhanced in the absence of HCN1 channels or by inhibition of I_h, corroborating that presynaptic I_h limits release in this region [58]**. Interestingly, whilst post-synaptic HCN1 subunit expression in the mEC was regulated by TRIP8b, presynaptic HCN1 subunit localisation and function were unaffected by TRIP8b [59]. This strongly suggests that pre- and post- synaptic HCN1 channels localisation and function are independently regulated, raising the exciting possibility that pre- and post- synaptic HCN channels may differentially affect neural network excitability.

Interestingly, in the calyx of held, HCN channels serve to regulate Na^+ concentrations within synaptic terminals [60]**. Vesicles within terminals express a Na^+/K^+ exchanger which facilitates glutamate uptake into vesicles. Inhibition of HCN channels results in less Na^+ influx within the terminal, reduced glutamate uptake into the vesicle and thereby

decreased glutamate release [60]**. Hence, the effects of I_h on synaptic release are very much dependent upon the type of terminal and the additional ion channels and cellular proteins that are present within the terminal.

In addition to synaptic terminals, HCN channels may also be expressed in axons and axon initial segments (AIS). HCN channels expressed in the AIS of mesial superior olive (MSO) principal neurons [61]** and dentate gyrus parvalbumin-positive interneurons [62] critically influence firing patterns of these neurons. In MSO neurons, modulation of these channels by serotonin (5-hydroxytryptamine (5-HT)) acting on 5-HT_{1A} receptors leads to long-lasting inhibition of these channels, strongly suggesting that neurotransmitters may modify presynaptic HCN channel activity too [61]**. Thus, like in somato-dendritic compartments, presynaptic I_h leads to diverse effects throughout the CNS which may have substantial effects on neuronal and network excitability.

Concluding Remarks

To sum up, HCN channels are cation channels that are activated by hyperpolarization. Their expression varies throughout the CNS, with many principal neurons expressing these channels in somato-dendritic compartments. Here, they influence the RMP and membrane resistance. Subsequently, these channels impact synaptic potential shapes and integration, neuronal activity as well as synaptic plasticity. In this manner, HCN channels affect physiological states such as learning and memory as well as pathophysiological conditions such as epilepsy.

Intriguingly, emerging evidence suggests that HCN channels are also present presynaptically in select inhibitory and excitatory neuronal axons and synaptic terminals. Here, they regulate neuronal activity and synaptic release in diverse ways in a synapse- and cell-specific manner. This together with the receptor-dependent and activity-dependent alterations in their function may lead to distinct ways in which these channels may alter neuronal and network function in the CNS.

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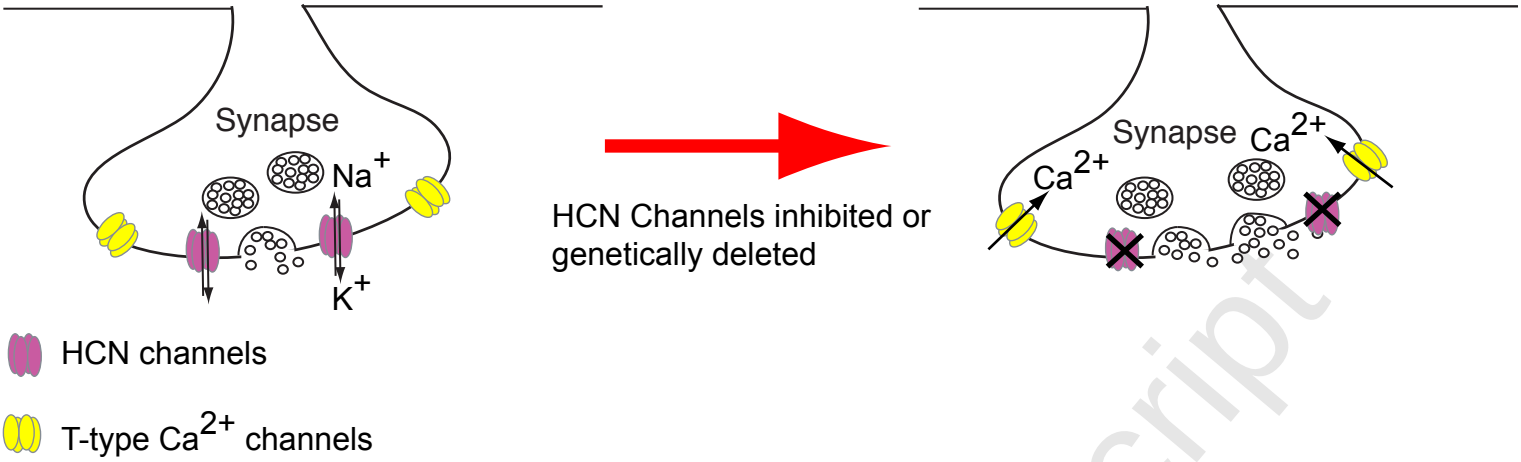
Figure Legends

Fig 1: HCN channels modify intrinsic membrane properties, excitatory postsynaptic potential (EPSP) shapes and integration as well as EPSP-spike coupling. (i) Morphology of an entorhinal cortical (EC) layer III pyramidal neuron. Scale bar = 50 μm (ii) Typical traces obtained when a 100 pA hyperpolarizing pulse was applied to wildtype (wt) and HCN1 null ($HCN1^{-/-}$) EC layer III dendrites from a fixed potential of -70 mV, demonstrating that the input resistance of $HCN1^{-/-}$ dendrites is much greater than wildtypes. (iii) Single and trains of simulated EPSPs recorded from $HCN1^{-/-}$ and wildtype dendrites at the common potential of -70 mV in response to alpha waveform injections. (iv) Example recordings obtained from the soma of $HCN1^{-/-}$ and Wt neurons at a fixed potential of -75 mV when 5 stimuli were applied extracellularly at 50 Hz to distal dendrites in the presence of $GABA_A$ receptor inhibitors. The stimulus strength was adjusted so that the amplitude of the EPSP generated by the first stimulus was 1-2 mV. The panel on the right shows the average somatic membrane potential of Wt and $HCN1^{-/-}$ neurons at which a train of 5 EPSPs generated by extracellular stimulation produced an action potential. The black and red dotted lines indicate the average normal resting membrane potential for Wt and $HCN1^{-/-}$ neurons respectively. The numbers of recordings obtained for each point are shown in parenthesis. *Adapted from Huang et al. (2009) J. Neurosci., **29**, 10979-88.*

Fig 2: HCN1 channels restrict synaptic release at select medial entorhinal cortical synapses. Schematic to show that HCN1 channels present in synapses limit glutamate release by restricting Ca^{2+} entry via voltage-gated T-type Ca^{2+} channels. HCN1 channels are normally active at rest and depolarize the synaptic terminal resting membrane potential (RMP). Inhibition of these channels hyperpolarizes the RMP and relieves the inactivation of T-type Ca^{2+} channels, thereby enhancing basal Ca^{2+} influx into the terminals and boosting spontaneous release. Adapted from *Huang et al. (2011) Nat.Neurosci., **11**, 478-86.*

Shah, 2017; Figure 2



Shah, 2017. Fig 1