Mechanisms of HCN channel trafficking and surface expression
Noam, Y.Y.

Citation for published version (APA):
Noam, Y. Y. (2011). Mechanisms of HCN channel trafficking and surface expression

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General Introduction

Parts of this chapter were published as a review article:

Towards an Integrated View of HCN Channel role in Epilepsy
Yoav Noam, Christophe Bernard & Tallie Z. Baram

Preface: voltage-gated ion channels are critical components in the maintenance and dynamic regulation of neuronal excitability

All brain functions rely on the ability of neurons to receive signals and respond to them. The combined effect of input and output in populations of neurons gives rise to complex patterns of network activity that govern our ability to think, learn, feel, move, and breathe. The propensity of a neuron to respond to a given input is termed neuronal excitability, and plays pivotal roles in the integration of neuronal signaling. Excitability is a highly regulated feature which needs to address two, seemingly conflicting demands: on the one hand, a certain degree of flexibility is required to ensure performance in changing contexts and to support processes of learning and memory. On the other hand, a robust system of checks and balances is required in order to constrain excitability within safe limits and thus prevent the system from reaching extreme situations of hypo- or hyper-excitability (Beck and Yaari, 2008; Noam and Baram, 2010).

Among the many factors that shape neuronal characteristics, voltage-gated ion channels play critical roles in the maintenance and regulation of excitability (Zhang and Linden, 2003; Beck and Yaari, 2008). A vast spectrum of ion channels co-exist in a neuron (Lai and Jan, 2006; Nusser, 2009), enabling fine-tuning of properties such as the membrane potential, input resistance, and intracellular ion homeostasis. These properties, in turn, facilitate critical neuronal functions including firing patterns, synaptic integration, presynaptic vesicle release, and intrinsic oscillations. Thus, it is the coordinated function of a variety of ion channels that shapes (to a large extent) the activity patterns of a neuron, and eventually - of the whole network. Excitability is controlled and modulated by processes that influence the abundance, sub-cellular localization, and functional properties of ion channels (Zhang and Linden, 2003; Nusser, 2009). Uncovering these regulatory processes is therefore important for increasing our understanding of excitability. In addition, this information is crucial for understanding pathological conditions in which such regulatory mechanisms fail to constrain neuronal excitability.

The work presented in this thesis aims to identify and elucidate mechanisms that dynamically regulate the trafficking, surface expression and biophysical properties of a unique sub-class of ion channels known as “HCN channels”. While these curious membrane proteins play versatile and important roles in modulation of neuronal function, the precise mechanisms that underlie their regulation are not understood.
HCN channels: A unique class of ion channels, mediating the hyperpolarization-activated current $I_h$

The **Hyperpolarization-activated, Cyclic Nucleotide-gated (HCN)** channels are unique among voltage-gated cationic channels due to their activation upon relative hyper- (rather than de-) polarization of the cell membrane (Robinson and Siegelbaum, 2003; Biel et al., 2009). Although these channels are structurally homologous to members of the potassium-channel superfamily (Fig. 1A), their functional properties are quite different: upon opening, HCN channels mediate a mixed cationic current ($I_h$), which slowly activates but does not inactivate (Fig. 1B). With reversal potential values between -30 and -40 mV, activation of $I_h$ under physiological conditions results in relative depolarization of the cell membrane, which counteracts the hyperpolarization that evoked it (Fig. 1C).

![Figure 1 - Basic structural and functional properties of HCN channels.](image)

One of the hallmarks of $I_h$ is its remarkable diversity in biophysical properties across different cell types and brain regions. For example, the time constants of channel activation may vary between ~30 milliseconds and a few seconds, and the half-activation voltage ($V_{50}$) values can range between -60 and -90 mV (Robinson and Siegelbaum, 2003). How is this heterogeneity...
obtained? One critical determinant is the molecular make-up of HCN channels. Four different channel isoforms exist (HCN1-4) that can assemble in different combinations to yield homo- or hetero-tetrameric complexes with different properties (Fig. 2) (Robinson and Siegelbaum, 2003; Santoro and Baram, 2003; Biel et al., 2009). Indeed, variability in $I_h$ properties among cell populations and during different developmental stages is often associated with distinct expression profiles of specific HCN channel isoforms (Kuisle et al., 2006; Brewster et al., 2007; Bender and Baram, 2008; Kanyshkova et al., 2009). The conductive properties of HCN channels are further diversified by discrete interactions with other molecules, notably cyclic AMP (cAMP): cAMP binds a sequence on the C’ terminus of the channel, and influences HCN channel function by accelerating its kinetics and shifting its voltage-activation curve to more depolarized values (Robinson and Siegelbaum, 2003; Santoro and Baram, 2003; Biel et al., 2009). The sensitivity of HCN channels to cAMP is isoform-specific: HCN4 > HCN2 >> HCN1 (Robinson and Siegelbaum, 2003; Santoro and Baram, 2003; Biel et al., 2009). Thus, isoform-specific interaction of HCN channels with regulating molecules provides another dimension to the modulation of $I_h$. HCN channel function is further modified by phosphorylation (Li et al., 2008; Liao et al., 2010; Hammelmann et al., 2011), as well as through interactions with PIP(2) (Zolles et al., 2006; Pian et al., 2007) and additional interacting/auxiliary proteins, which will be discussed later on in this chapter.

**Figure 2 - HCN channels are tetramers of either hetero- or homo-meric composition.**
Functional HCN channels are tetramers, and different HCN channel subunits (HCN1-4) can assemble together to form either homomeric or heteromeric channels, with distinct biophysical properties. A schematic representation of homomeric and heteromeric HCN1 and HCN2 channels (the most abundant HCN subunits in the adult hippocampus) is shown.
Chapter 1

The diverse roles of HCN channels and \( I_h \) in neuronal excitability

In line with its diverse biophysical properties discussed above, the roles of \( I_h \) in regulating neuronal excitability are numerous and complex. Alongside its depolarizing effect on membrane potential, \( I_h \) exerts an additional, shunting effect on excitable cells: by being open at sub-threshold potentials, \( I_h \) reduces the input resistance of the membrane (\( R_{in} \)), thus dampening the ability of incoming inputs to alter membrane voltage.

Active at sub-threshold potentials, \( I_h \) is particularly suited for the regulation of intrinsic excitability. Membrane conductances interact in a highly non-linear way through membrane voltage, so that \( I_h \) modulation in different physiological contexts may lead to different outcomes. For example, the net effect of the depolarizing and shunting properties of \( I_h \) on excitability is combinatorial and depends on many factors (Fig. 3), as illustrated by the roles of \( I_h \) in CA1 hippocampal pyramidal neurons. In these neurons, the density of \( I_h \) along apical dendrites increases with the distance from the soma (Magee, 1999). A physiological consequence of this heterogeneous distribution (mediated by the effect of \( I_h \) on the input resistance and membrane time constant) is that EPSP time course is increasingly shortened with the distance from the soma (Magee, 1999). As a result, temporal summation of synaptic inputs at the soma is similar regardless of whether these inputs originate at proximal or distal sites (Magee, 1999; but see Angelo et al. 2007). Another physiological action of \( I_h \), mediated by its tonic depolarizing effect, is to increase steady-state inactivation of low-threshold, voltage-gated calcium channels (Tsay et al., 2007), and thus restrict the genesis of dendritic calcium spikes. The effects of \( I_h \) on excitability involve interactions with other intrinsic conductances: whereas \( I_h \) increases the peak voltage amplitude of weak EPSPs, it inhibits the peak amplitude of responses to strong stimuli, and the net effect on EPSPs depends upon an intricate interaction between the \( I_h \)–mediated tonic depolarization and the potassium-conducting current, \( I_m \) (George et al., 2009). Regulation of synaptic signaling by \( I_h \) is not limited to excitatory input, and \( I_h \) suppresses inhibitory (GABA\(_A\)-mediated) post-synaptic potentials (IPSPs) through interactions with other active conductances and cellular passive electric properties (Williams and Stuart, 2003; Hardie and Pearce, 2006). The relative depolarization generated by \( I_h \) in distal dendrites can alter GABA\(_A\) signaling from shunting to hyperpolarizing, and thus shortens the time-window for coincidence-detection (Pavlov et al., 2011).

From the above, it is clear that \( I_h \) has multiple effects in regulating dendritic excitability, including through interactions with other intrinsic conductances. Yet how do these different effects of \( I_h \) on inhibitory, excitatory and intrinsic signals integrate in a physiological context? This question was recently addressed in subthalamic nucleus (STN) neurons (Atherton et al.,
In these neurons (which express different HCN channel subunits than CA1 pyramidal neurons), dendritic $I_h$ was activated only upon strong hyperpolarizing input onto the dendrite, and thus served a homeostatic role in counteracting GABA$_A$-mediated signals. In an analogous manner to that described in CA1 pyramidal neurons (Tsay et al., 2007), activation of $I_h$ by inhibitory input facilitated steady-state inactivation of low-threshold voltage-gated calcium channels, leading to inhibition of dendritic calcium spikes. Whereas theoretically $I_h$ could suppress also the temporal summation of excitatory inputs, the authors demonstrated that the same inhibitory input that activated $I_h$ shunted the effects of $I_h$ on EPSP summation,
and concluded that, in STN neuronal dendrites, $I_h$ selectively regulated inhibitory signaling (Atherton et al., 2010).

$I_h$ is not limited to somato-dendritic subcellular distribution, and presynaptic $I_h$ has been reported in various classes of neurons (Luján et al., 2005; Aponte et al., 2006; Bender et al., 2007; Huang et al., 2011). The presynaptic functions of $I_h$ in mammalian brain have remained elusive (Bender et al., 2007; Biel et al., 2009), but, in axon terminals of layer 3 entorhinal cortex neurons, $I_h$-mediated depolarization was recently found to restrict the activity of T-type $\text{Ca}^{2+}$ channels, leading to reduced calcium influx and inhibition of synaptic release (Huang et al., 2011). It remains to be seen whether similar mechanisms exist in presynaptic terminals of interneurons (Luján et al., 2005; Aponte et al., 2006) or whether $I_h$ serves an opposite, facilitating role in these neuronal populations (Luján et al., 2005).

In addition to its roles in dendritic integration, membrane potential stabilization and regulation of synaptic transmission, $I_h$ is an important modulator of oscillatory activity at both cellular and network levels. At cellular levels, $I_h$ is critical for theta resonance (the preferential response of a neuron to oscillating inputs at specific frequencies). The slow kinetics and sub-threshold activation of $I_h$ enable this current to filter out inputs at low frequencies (<3 Hz). This high-pass filtering property, in combination with low-pass filtering properties provided by the membrane capacitance, render cells with a strong $I_h$ particularly responsive to inputs in the theta range (3-12 Hz) (Narayanan and Johnston, 2007; Hu et al., 2009), a property also found in specific types of interneurons (Zemankovics et al., 2010). In cell-types with high $I_h$ density at distal dendrites, dendritic $I_h$-dependent resonance is more pronounced, and preferentially filters signals that propagate from the soma to dendrites (Hu et al., 2009). The filtering properties of $I_h$ may contribute to regulating in vivo theta rhythms (Nolan et al., 2004), and other network oscillations (e.g., delta and gamma) and rhythmic firing (reviewed by Biel et al., 2009).

**Dynamic regulation of $I_h$**

In addition to the spatial diversity of $I_h$ across brain tissues and cell types, temporal and activity-dependence of $I_h$ contributes to the dynamic regulation of neuronal excitability. In hippocampal CA1 pyramidal neurons, a substantial up-regulation of $I_h$ amplitude occurs within minutes following the enhancement of excitatory input induced by either glutamate application or by theta-burst pairing of Schaffer collaterals (van Welie et al., 2004; Fan et al., 2005). Activity-dependent regulation of $I_h$ plays a homeostatic role in the regulation of intrinsic excitability (Fig. 4): by lowering the membrane input resistance, increased $I_h$ dampens the ability of incoming inputs to depolarize the membrane potential and therefore
decreases their probability of triggering an action potential (van Welie et al., 2004). The true homeostatic nature of $I_h$ regulation is further demonstrated by the down-regulation of $I_h$ following long-term depression (LTD), implying a bi-directional mechanism by which $I_h$ either enhances or reduces intrinsic excitability in response to the excitable state of the network (Brager and Johnston, 2007). Comparing between different forms of LTP in CA1 pyramidal neurons revealed the intricate, complex nature of $I_h$-dependent plasticity: whereas “strong” activation of the Schaffer collaterals (using theta burst stimulation) leads to an up-regulation of $I_h$ (Fan et al., 2005; Campanac et al., 2008) weaker LTP protocols such as high-frequency stimulation or spike-timing-dependent plasticity induce down-regulation of $I_h$ (Campanac et al., 2008). Thus, $I_h$ may function as a sensitive tuner of intrinsic excitability: upon strong fluctuations in excitatory input, $I_h$ may act to restore intrinsic excitability in a homeostatic manner (thus “pushing” the shifted excitability back to its original set-point), while upon weaker alterations - $I_h$ may actually contribute to the shift of neuronal excitability away from its original set-point.

Despite their physiological relevance, little is known about the cellular and molecular mechanisms that underlie activity-dependent modulation of $I_h$. Activation of both AMPA- and NMDA- type ionotropic glutamate receptors is required for the activity-dependent up-regulation of $I_h$, as well as increased levels of intracellular calcium (van Welie et al., 2004; Fan et al., 2005). Insertion (or removal) of HCN channels within the cell membrane is an intriguing mechanism by which neuronal-activity may dynamically control the properties and magnitude of $I_h$. Mechanisms of membrane insertion and internalization are ideally suited for plasticity processes in the time-scale of minutes, allowing for rapid responses in membrane excitability. Indeed, the involvement of membrane trafficking in neuronal plasticity has been established for many ion channels, including ionotropic glutamate receptors (Newpher and Ehlers, 2008), GABA$_A$ receptors (Luscher et al., 2011), and various voltage gated ion channels (Jarvis and Zamponi, 2007; Jensen et al., 2011). It is not yet clear whether dynamic regulation of HCN channel trafficking and surface expression is involved in $I_h$-related plasticity.
Figure 4 - Homeostatic regulation of intrinsic excitability by $I_h$. A. Enhancement of excitatory input by means of glutamate application to the soma augments $I_h$ amplitude within minutes, as demonstrated by cell-attached recordings from CA1 hippocampal neurons (van Welie et al., 2004). B. up- and down-regulation of $I_h$ can influence intrinsic excitability by shifting the input-output curve. Enhancement of $I_h$ dampens the input resistance of the membrane. With lowered input resistance, stronger stimuli are required in order to produce action potentials. Reduction of $I_h$ is expected to work in the opposite direction by increasing input resistance, thus rendering the cell more likely to fire in response to an incoming input. The activity-dependent up- or down-regulation of $I_h$ may thus result in the homeostatic scaling of intrinsic excitability.
Emerging roles for auxiliary proteins in regulating HCN channel trafficking and function

The highly versatile nature of HCN channel expression, distribution, and function requires tight level of regulation, and interaction with auxiliary subunits may provide a potent way to regulate such properties. Indeed, important roles for auxiliary proteins have been implicated in regulating a myriad of ion channels, including glutamate receptors (Jackson and Nicoll, 2011), potassium channels (Vacher and Trimmer, 2011), and voltage-gated calcium channels (Dolphin, 2009). Since the original cloning of HCN channels in 1998 (Santoro et al., 1998), several auxiliary proteins that directly bind to specific HCN channel isoforms have been identified (Table 1). However, the functional influence and physiological relevance of many of these proteins remain poorly understood. Of the various candidates, the HCN-interacting protein TRIP8b is particularly intriguing: TRIP8b is a brain-specific HCN auxiliary subunit which is distributed in a gradient-like fashion along the dendrites of CA1 pyramidal neurons, resembling the distribution of HCN channels in these neurons (Santoro et al., 2004). Recently, we and others have discovered a family of TRIP8b splice isoforms. Interestingly, whereas distinct TRIP8b isoforms could either up- or down-regulate surface expression of the channels, all TRIP8b isoforms inhibited channel gating (Lewis et al., 2009; Santoro et al., 2009).

Another promising candidate for HCN channel regulation is filamin A (FLNa), a cytoskeletal protein that stabilizes the actin network by crosslinking actin filaments (Stossel et al., 2001; Popowicz et al., 2006). In addition to its structural roles, FLNa binds to a variety of membrane proteins and influences their trafficking and stability within the membrane (Stossel et al., 2001; Popowicz et al., 2006). In melanoma cell lines, direct interaction of FLNa with HCN1 channels inhibited $I_h$ amplitude and gating, and altered the distribution of HCN channels on the cell surface (Gravante et al., 2004). It is unclear whether similar processes occur in the central nervous system. In fact, very little is known on the presence and role of FLNa in the adult brain: work in the field has focused so far on early developmental stages, when FLNa is critical for neuronal migration and cortical maturation (Sarkisian et al., 2008). Whereas FLNa may serve key roles in the regulation of HCN channels within the cytoskeletal scaffold, the mechanisms by which it influences channel trafficking and surface expression are currently unknown. Thus, unraveling the discrete mechanisms by which auxiliary proteins (such as TRIP8b and FLNa) control HCN channel function and trafficking is critical for our understanding of the modulation of these channels and may provide insights into their dynamic regulation, which underlies various forms of plasticity.
<table>
<thead>
<tr>
<th>Protein</th>
<th>HCN</th>
<th>Functional Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIP8b</td>
<td>HCN1,2,4</td>
<td>Hyperpolarizing shift of the voltage-activation profile and slowed activation kinetics.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Up- or down-regulation of HCN surface expression and I(_h) amplitude (differs between specific splice isoforms).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two interaction sites: (1) *SNL aa sequence at the C' terminus, and (2) a binding region at the cyclic nucleotide binding domain of the channel.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experimental system: HEK293, oocytes, hippocampal neurons, hippocampal slices.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Santoro et al., 2004; Lewis et al., 2009; Santoro et al., 2009)</td>
</tr>
<tr>
<td>Filamin A</td>
<td>HCN1 (but not HCN2,4)</td>
<td>Decreased I(_h) amplitude, slower activation kinetics, hyperpolarizing shift of the voltage-activation profile, and altered distribution on the cell surface.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 aa sequence at the C' terminus of HCN1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A sequence on the C' terminus of HCN2,3,4 (not tested for HCN1).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experimentally confirmed and the channel isoform studied and the experimental system used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Gravante et al., 2004)</td>
</tr>
<tr>
<td>KCNE2/MiRP1</td>
<td>HCN1,2,4</td>
<td>Conflicting effects on I(_h) amplitude, kinetics and voltage-activation were reported, depending on the HCN channel isoform studied and the experimental system used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A sequence on the C' terminus of HCN2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Yu et al., 2001; Proenza et al., 2002; Altomare et al., 2003; Decher et al., 2003; Qu et al., 2004; Brandt et al., 2009)</td>
</tr>
<tr>
<td>KCR1</td>
<td>HCN2</td>
<td>Suppression of whole-cell I(_h) amplitude and single-channel properties.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyperpolarizing shift of the voltage-activation profile.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Michels et al., 2008)</td>
</tr>
<tr>
<td>Tamalin</td>
<td>HCN2 (but not HCN1,4)</td>
<td>Unknown binding domain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Kimura et al., 2004)</td>
</tr>
<tr>
<td>S-SCAM</td>
<td>HCN2</td>
<td>Unknown binding domain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Kimura et al., 2004)</td>
</tr>
<tr>
<td>Mint2</td>
<td>HCN2</td>
<td>Unknown binding domain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>References: (Kimura et al., 2004)</td>
</tr>
</tbody>
</table>

Table 1: HCN-interacting proteins

*For review, see Lewis et al., 2010
Scope and aim of this thesis

To fully grasp the mechanisms by which $I_h$ controls excitability in diverse contexts, further insight into the dynamics and regulation of HCN channels is required. The work presented in this thesis aims at investigating mechanisms that control HCN channel trafficking and surface expression in neurons. More specifically, I focus on two key questions:

1. Do trafficking and/or surface expression of HCN channels play a role in the activity dependent regulation of $I_h$?

2. What is the role of HCN-auxiliary proteins in regulating HCN channel trafficking and function, and what are the cellular and molecular mechanisms that underlie such regulation?

In chapter 2, a live-imaging approach is employed to directly visualize and characterize the dynamics of HCN channel trafficking in dendrites of hippocampal neurons, and to investigate their activity-dependent properties. Chapter 3 provides an in-depth inquiry into the distinct mechanisms and molecular interactions by which the auxiliary protein TRIP8b regulates HCN channel trafficking and $I_h$ biophysical properties. In chapters 4 and 5, we study the potential roles of the cytoskeletal protein filamin A in controlling HCN channel trafficking, surface expression and function. To that aim, a thorough characterization of FLNa expression and subcellular distribution in the adult brain is first undertaken (Chapter 4), followed by studies into the cellular mechanisms that underlie regulation of HCN channel trafficking and surface expression by FLNa (Chapter 5). Chapter 6 provides a general discussion, highlighting intriguing hypotheses and future research directions in the study of HCN channel trafficking, based on the evidence provided by the experiments described in this thesis.
Chapter 1

Box 1: HCN channel dysregulation in epilepsy: The wrong amount in the wrong place, at the wrong time

Epilepsy is a severe brain pathology where compromised regulation of excitability results in the spontaneous occurrence of seizures. In line with the multiple roles of HCN channels in regulating excitability, many studies have linked these channels to the epileptogenic process. In resected hippocampi from patients with temporal-lobe (limbic) epilepsy, enhanced levels of HCN1 channel expression and dendritic localization were found in granule cells of the dentate gyrus (Bender et al., 2003), and recent work has identified a mutation in the \textit{HCN2} gene and augmentation of \textit{I}_h in patients with genetic epilepsy with febrile seizures plus (GEFS+) (Dibbens et al., 2010). Deletion of the \textit{HCN1} gene in mice results in increased excitability and seizure susceptibility (Huang et al., 2009; Santoro et al., 2010), and reduction or deletion of the \textit{HCN2} isoform leads to spontaneous ‘absence’ seizures (Ludwig et al., 2003; Chung et al., 2009b).

In accord with the diverse regulatory mechanisms and versatile functions of \textit{I}_h in the normal brain, the \textit{dysregulation} of \textit{I}_h and HCN channels in epilepsy is dynamic and intricate (Fig. 5). HCN channel abnormalities in the epileptic brain can manifest as altered mRNA and protein expression (Brewster et al., 2002; Shah et al., 2004; Brewster et al., 2005; Jung et al., 2007; Powell et al., 2008; Budde et al., 2005), sub-cellular distribution (Shin et al., 2008; Hablitz and Yang, 2010) or biophysical properties (Chen et al., 2001a; Jung et al., 2007; Dyhrfjeld-Johnsen et al., 2008). The causal relationship between aberrant HCN channel regulation and the epileptic process is further complicated by the fact that alterations in HCN channel expression, localization and function vary across animal models of epilepsy. Both early and late changes affecting diverse isoforms in distinct spatial patterns have been reported. For example, in the pilocarpine model, progressive reduction in HCN1 and HCN2 protein levels results in diminished \textit{I}_h amplitude in dendrites of CA1 pyramidal neurons, leading to the disruption of theta resonance (Jung et al., 2007; Marcelin et al., 2009). In contrast, following hyperthermia-induced seizures, the same population of cells exhibit \textit{enhanced} dendritic \textit{I}_h accompanied by altered gating properties (Chen et al., 2001a; Dyhrfjeld-Johnsen et al., 2008), likely mediated by isoform-specific transcriptional regulation and increased HCN1/HCN2 heteromerization (Brewster et al., 2005). These seemingly conflicting results from different animal models demonstrate that augmented \textit{I}_h can be associated with both increased and decreased excitability, depending on the physiological context (Dyhrfjeld-Johnsen et al., 2009), and potential interaction with other conductances.
The *temporal* patterns of HCN dysregulation in the epileptic brain are complex, and both transient and long-lasting alterations in hippocampal HCN/I\(_h\) have been reported in pilocarpine, KA and febrile seizures models (Brewster et al., 2002; Shah et al., 2004; Brewster et al., 2005; Jung et al., 2007; Marcelin et al., 2009). Interestingly, alterations in I\(_h\) gating properties in CA1 pyramidal neurons were reported within hours following induction of seizure-like-activity *in vitro*, attributable to activation of the phosphatase calcineurin and inhibition of p38 MAPK (Jung et al., 2010).

Spatial selectivity of HCN-channel pathology further contributes to the complex involvement of I\(_h\) in epilepsy. In acquired hippocampal epilepsy, HCN channel expression levels vary across different hippocampal regions in isoform-specific and region-specific fashions (Brewster et al., 2002; Powell et al., 2008). Region-specific alterations have been found in the WAG/Rij rat model of genetic absence epilepsy, where *reduced* HCN1 channel expression in layer 5 cortical neurons was reported to increase excitability via enhanced calcium electrogenesis (Kole et al., 2007), while *increased* HCN1 levels in thalamocortical neurons impaired their firing pattern via reducing the responsiveness of I\(_h\) to cAMP (Budde et al., 2005). Reduced response of I\(_h\) to cAMP (triggered by imbalance of HCN subunit expression) was found also in the GAERS rat model of absence epilepsy (Kuisle et al., 2006).

Epilepsy may also involve abnormal subcellular distribution of HCN channels. Dendritic HCN1 localization was augmented in hippocampi resected from patients with epilepsy (Bender et al., 2003). In animal models, transient upregulation of HCN1 surface expression occurred in CA1 pyramidal neurons 1-2 days after an epilepsy-provoking insult, followed by a *down*regulation 4 weeks later with mislocalization of the channels from distal dendritic domains to somata (Shin et al., 2008). Recent work in freeze-lesion models of cortical dysplasia-provoked epilepsy found reduced HCN1 channel presence in distal dendrites of layer 5 cortical neurons (Hablitz and Yang, 2010).

In summary, multiple mechanisms, including transcriptional control, trafficking and channel modification act at different temporal and spatial scales to modulate the epileptic brain. In general, such changes might contribute to epileptogenesis, i.e., ‘cause’ epilepsy, or be a result of the epilepsy. The occurrence of several of these HCN changes early after the insult that triggers epilepsy, and/or prior to the onset of spontaneous seizures (Kole et al., 2007; Brewster et al., 2002; McClelland, 2011) suggests a causal role. However, to fully grasp the contribution of these alterations to the epileptogenic process, physiological, molecular and cellular approaches should be integrated.
Figure 5 - Abnormal HCN channel regulation and function in epilepsy. Dysregulation of HCN channels/ I$_h$ in epilepsy occurs at multiple levels. Seizure-induced alterations of the biophysical properties of I$_h$ include either upregulation or downregulation of current amplitude and modification of gating. The subcellular distribution of HCN channels along the somato-dendritic axis is altered both short-term, for example, in hippocampal CA1 neurons in the pilocarpine model and in dentate gyrus granule cells of human epileptic patients. Seizure-induced alterations in HCN channel mRNA and protein levels occur in many epilepsy models, and are both region-specific and channel isoform-specific. Altered synthesis of specific HCN channel isoforms may drive, at least partly, the increased, seizure-induced HCN1/2 heteromerization in hippocampus (Brewster et al., 2002; 2005). Insets were modified from Brewster et al., 2002, Bender et al., 2003, and Shin et al., 2008.