THE ROLE OF $I_H$ IN 4-AMINOPYRIDINE INDUCED EPILEPTIFORM ACTIVITY

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ABSTRACT

Our laboratory has taken a recent interest in understanding the role $I_h$ plays in epilepsy. $I_h$ is an inward rectifying current which is activated at hyperpolarized membrane potentials. Our lab found that in the freeze lesion epilepsy model, $I_h$ is reduced, EPSP summation is increased, and membrane excitability is increased. In the present study, we used whole cell patch clamp technique to examine the role of $I_h$ in the 4-AP hyperexcitability model. We first wanted to establish the effects of 4-AP on layer 5 (L5) pyramidal neurons. Treatment of L5 pyramidal cells with 4-AP resulted in changes in the intrinsic properties of the cell, but did increase the network excitability signified by the generation of evocable epileptiform discharges. Next, we examined the effects of $I_h$ inhibition on the intrinsic membrane excitability and network excitability in L5 pyramidal cells. Using somatic current-clamp recordings, we found that inhibition of $I_h$ with ZD7288 resulted in increased excitability of L5 pyramidal cells. Inhibition of $I_h$ increased the durations of evoked epileptiform discharges and increased the duration of inhibition. Voltage clamp analysis revealed that inhibition of $I_h$ resulted in changes in the early excitatory current as well as increase in the duration of epileptiform discharges. $I_h$ inhibition resulted in depolarizing shift in the reversal potential of the early excitatory current of the epileptiform discharge as well as a decrease in membrane conductance.

This study also examined the effects enhancing $I_h$ would have on intrinsic excitability and network activity in the 4-AP model. Once again using somatic current-
clamp recordings, it was shown that enhancing $I_h$ with lamotrigine lead to a decrease in
the intrinsic excitability of L5 pyramidal cells. Enhancement of $I_h$ caused decreases in
the duration of inhibition as well as decreases in maximum amplitude and duration of
epileptiform events. Voltage clamp analysis revealed decreases in the duration and
maximum amplitude of the epileptiform discharges following the enhancement of $I_h$.
Enhancement of $I_h$ also caused a decrease in the early excitatory conductance, but to
alterations in the reversal potential. Taken together, these experiments reveal that in a 4-
AP induced hyperexcitable cortical network, alterations in $I_h$ results in changes in both
intrinsic and network excitably. The epileptiform discharges observed in this study were
GABA mediated and influenced by $I_h$. Thus, the resultant $I_h$ mediated changes in
network excitability were in part due to its effects on synaptic GABAergic signaling.
Overall, alterations in $I_h$ affected both network and intrinsic excitability of L5 pyramidal
cells in the 4-AP hyperexcitability model.

Keywords: epileptiform discharge, 4-AP, ZD7288, lamotrigine, $I_h$
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<td>4-AP</td>
<td>4-Aminopyridine</td>
</tr>
<tr>
<td>aCSF</td>
<td>artificial cerebral spinal fluid</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>calcium</td>
</tr>
<tr>
<td>EPSC</td>
<td>excitatory postsynaptic current</td>
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<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>GABA_A</td>
<td>gamma-aminobutyric acid type A</td>
</tr>
<tr>
<td>GABA_B</td>
<td>gamma-aminobutyric acid type B</td>
</tr>
<tr>
<td>HCN</td>
<td>Hyperpolarization-activated Cyclic Nucleotide-gated Channel</td>
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<tr>
<td>I_A</td>
<td>A-type potassium channel</td>
</tr>
<tr>
<td>I_D</td>
<td>D-type potassium channel</td>
</tr>
<tr>
<td>I_h</td>
<td>HCN channel associated Current</td>
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<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>IPSC</td>
<td>inhibitory postsynaptic current</td>
</tr>
<tr>
<td>K^+</td>
<td>potassium</td>
</tr>
<tr>
<td>L5</td>
<td>layer 5</td>
</tr>
<tr>
<td>pA</td>
<td>picoamps</td>
</tr>
<tr>
<td>MΩ</td>
<td>megaohms</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>------------------------------------------------</td>
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<tr>
<td>μS</td>
<td>microsiemens</td>
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<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>mV</td>
<td>millivolts</td>
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<tr>
<td>NMDA</td>
<td>(N)-methyl-D-aspartate</td>
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<tr>
<td>PSC</td>
<td>postsynaptic current</td>
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<tr>
<td>PSP</td>
<td>postsynaptic potential</td>
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<tr>
<td>SEM</td>
<td>standard error mean</td>
</tr>
<tr>
<td>ZD7288</td>
<td>4-Ethylphenylamino-1,2-dimethyl-6-</td>
</tr>
<tr>
<td></td>
<td>methylaminopyrimidinium chloride</td>
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Introduction

Studying Epilepsy

According to the American Epilepsy Foundation, almost 3 million people in the United States and 70 million people worldwide have some form of epilepsy (Hirtz et al., 2007, Ngugi et al., 2011). The incidence of epilepsy is highest in infants and the elderly. This neurological disease hampers or disrupts the afflicted’s daily life experiences for a significant population of humans (Leeman et al., 2008). The goal of treatment is to relieve patients of seizures without side effects and to return patients to a normal, healthy lifestyle.

A proportion of epileptic patients are resistant to currently available antiepileptic drugs (AED’s). There has also been an observed failure of anticonvulsants to control seizures in 30% of epileptic patients treated (Wahab et al., 2010). What can the epileptologists do to help this afflicted population? There is a lack of a knowledge concerning the cellular basis of epilepsy, so a better understanding of basic mechanisms of the processes leading to epilepsy needs to be gained to allow for the creation of therapies for at risk patients as well as aid in the development of disease-modifying therapies to inhibit the progression of the disease. Gaining more insight into the pathophysiology of the disease will also allow for an improved understanding of the observed pharmocoresistance thus allowing for the development of drugs for the reversal or prevention of resistance.
Epilepsy is a neurological disease characterized by chronic seizures due, in part, to dysfunction in the synaptic regulatory machinery. Epilepsy can be categorized by the seizure etiology, the seizure type, or the epilepsy syndrome. Epileptic syndromes can be categorized as either genetic, structural/metabolic, and of unknown cause with underlying damage or disease. Some of the epileptic syndromes include: seizure syndromes in newborns, febrile convulsions, juvenile myoclonic epilepsy, temporal lobe epilepsy, and frontal lobe epilepsy, amongst others. Each epileptic syndrome includes respectively unique regional variations in circuitry and cellular properties as well as ion channel dysfunction thus creating an environment which is susceptible for seizure induction.

Regardless of the etiology of the disease, epilepsy involves a neuronal hyperexcitability at the cellular and/or network level (McCormick and Contreras, 2001, Pitkänen and Lukasiuk, 2009).

One way to analyze the mechanism underlying epileptogenesis in humans is by recreating the pathophysiology in an animal model. This can be performed by mimicking the underlying mechanistic change in excitability observed in epilepsy through acute epilepsy models. In these models, seizures are induced via electrical or chemical/ionic stimulation in healthy, naïve animals (Pitkänen et al., 2005). The pharmacological models of epilepsy include 4-Aminopyridine (4-AP), kainic acid, picrotoxin, bicuculline-methiodide, pilocarpine, and low magnesium amongst others. These models are thought to induce epileptiform activity by altering different cellular mechanisms. Thus, by testing different antiepileptics through the various models, the anticonvulsant properties of the drugs can be illuminated.
The 4-AP Hyperexcitability Model

4-AP is a convulsant which is associated with prolonged action potentials and enhancement of synaptic potentials (Buckle and Haas, 1982, Perreault and Avoli, 1989, Gu et al., 2004, Wu et al., 2009). 4-AP been shown to induce seizures in humans and other mammals (Brückner et al., 1999). 4-AP is a K+ channel blocker which at varying concentrations can block delayed rectifier currents, transient inactivating A-type K+ currents (IA), and D-type K+ currents (ID), which are slowly inactivating K+ currents (Brückner et al., 1999). IA is known to be active at subthreshold membrane potentials to control the excitability of the cell. ID takes tens of seconds to recover from inactivation which enables the cell to integrate separate depolarizing inputs over long periods of time (Storm, 1988). 4-AP has been shown to augment presynaptic Ca²⁺ influx, increase neurotransmitter release, and increase extracellular K⁺ levels (Buckle and Haas, 1982, Rutecki et al., 1987, Chestnut and Swann, 1988, Perreault and Avoli, 1989, Perreault, 1991, Mattia et al., 1993, Flores-Hernández et al., 1994, Gu et al., 2004). The augmentation of presynaptic Ca²⁺ levels leading to an enhancement of neurotransmitter release is believed to be caused either by directly enhancing voltage-dependent Ca²⁺ channels or by prolonging the action potential by decreasing repolarizing K⁺ conductances (Perreault and Avoli, 1989).

The 4-AP hyperexcitability model enhances both excitatory and inhibitory neurotransmitter release. This increase in neurotransmitter release produces an epileptiform discharge that is multifaceted containing excitatory postsynaptic potential (EPSP), inhibitory postsynaptic potential (IPSP), and late IPSP components (Rutecki et al. 1987). The EPSP component has been observed to vary based on the animal species.
In guinea pig neocortical slices, the EPSP component is mediated by N-methyl-D-aspartic acid (NMDA) receptors (Mattia et al., 1993). In rat hippocampal slices, the EPSP component of this epileptiform discharge is mediated by non-NMDA or 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4yl)propanoic acid (AMPA) receptor (Mattia et al., 1993). The IPSP component of the epileptiform discharge is GABA_A-receptor mediated whereas the late IPSP has been linked to a K^+ conductance mediated by GABA_B receptors (Perreault and Avoli, 1989). The 4-AP model has also been linked to depolarizing GABA receptor mediated currents (Perreault and Avoli, 1992, Traub et al., 1995, Avoli, 1996, Sinha and Saggau, 2001, Isomura et al, 2008).

*In vitro* studies involving human tissue samples from patients with epileptic syndromes has shown 4-AP to have varied effects. The first study involved human neocortical slices from temporal lobe epilepsy patients none of which displayed any structural lesion or an extra-neocortical epileptic focus (Avoli et al, 1996). Negative going field potentials were observed in the presence of 4-AP as well as intracellular long-lasting depolarizations. These long-lasting depolarizations were accompanied by early and late long-lasting hyperpolarizations that were observed during the negative-going synchronous events. Another study used human neocortical slices from patients with focal neuronal migration disorders and observed a different pattern of activity (Avoli et al., 1996). This type of tissue is characterized by delamination with multifocal clustering of neurons that can be found in the white matter as foci. Both spontaneous and stimulus-induced seizure-like discharges were seen in the presence of 4-AP. It is interesting to note that the induced temporal lobe intracellular activity is similar to that induced in pyramidal and granule cells of rat hippocampal slices treated with 4-AP. When
bicuculline methiodide was bath applied onto entorhinal cortical slices, the 4-AP induced LLD’s were abolished revealing the occurrence of hyperpolarizations and prolonged epileptiform discharges (Brückner et al., 1999).

Characteristics of Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) Channels

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels generate a mixed cation (Na\(^+\)/K\(^+\)) current (I\(_h\)) under normal physiological conditions. I\(_h\) is non-inactivating with a reversal potential between -20 mV and -40 mV and is activated at hyperpolarized potentials (Lamas, 1998, DiFrancesco, 2010). I\(_h\) is mostly carried by Na\(^+\) due to a greater electrochemical gradient. Due to I\(_h\) being active at resting membrane potentials and mediated by Na\(^+\), there is a resulting depolarization of the resting membrane potential. Both the current amplitude and the ratio of Na\(^+\) to K\(^+\) permeability (P\(_{Na}\):P\(_K\)) depend on extracellular K\(^+\) concentration. An increase in extracellular K\(^+\) results in a reduced selectivity for K\(^+\) over Na\(^+\) as well as an increase in current amplitude (DiFrancesco, 1981, France, 1992, Ludwig et al., 1998). HCN channels have also been found to have a small but significant Ca\(^{2+}\) permeability (Yu et al., 2007).

HCN channels have been identified in tissues throughout the body (El-Kholy, 2007, Benham, et al., 1987, Green et al., 1996, Greenwood and Prestwich, 2002, Hisada et al., 1991, McCloskey et al., 1999, Okabe et al., 1999, Ouyang et al., 2007, Seifert et al., 1999, Xia et al., 2004, Yanagida et al., 2000, Zhang et al., 2007) with the highest expression levels being located in the brain and heart (Wahl-Schott and Biel, 2009). I\(_h\) is known to control rhythmic activity in neuronal circuits as well as contributing to neuronal resting membrane potentials, dendritic integration, and synaptic transmission (Wahl-
Schott and Biel, 2009). In neocortical and hippocampal pyramidal cells, HCN channel expression increases as distance from the soma increases (Lörincz et al., 2002). This increase in $I_h$ density in the dendrites leads to a decrease of the dendritic input resistance which leads to an inhibition of dendritic EPSP summation and EPSP-spike coupling (Dyhrfjeld-Johnsen, 2009). Thus, $I_h$ can influence excitability indirectly by altering the resting membrane potential and the tonic firing rate of the pyramidal cells.

**HCN Subunit Alteration and Disease**

In mammals, 4 homologous HCN subunits exist (HCN1-HCN4) (Wahl-Scott and Biel, 2009, Clampham, 1998). While splicing variants of the HCN isoforms have been observed in invertebrates (Giesselmann et al., 2004, Giesselman et al., 2005, Ouyang et al., 2009), splicing variants have not been observed in vertebrates. The channel is a tetramer consisting of heterologous or homologous isoform composition (Ludwig, 1998, Santoro and Tibbs, 1999). Each isoform conveys different kinetics and sensitivity to modulation by cAMP to the formed channel. The unique biophysical properties of each subunit were observed from the formation of homotetrameric channels. The homomeric HCN1 channels have the fastest kinetics for voltage-dependent activation while HCN4 has the slowest. HCN2 and HCN3 have activation kinetics in between HCN1 and HCN4. HCN2 has a cAMP activation dependence that is not seen in HCN1. HCN channels are expressed throughout the body with different isoform expression being higher in some areas than other (Santoro et al., 2000). With regards to the brain, HCN1 and HCN2 are the more common isoforms. HCN1 is expressed in the neocortex, hippocampus, cerebellar cortex, and brainstem. HCN2 is distributed nearly ubiquitously
throughout most brain regions while HCN3 is expressed at very low levels in the central nervous system and HCN4 being expressed at even lower levels than HCN3. In the heart, the $I_h$ current is known as $I_f$. The HCN1, HCN2, and HCN4 isoforms are expressed throughout the heart with expression levels vary depending on the age, species, disease state, and region of the cardiac tissue (Herrmann et al., 2007).

There is preferential cellular expression of HCN isoforms with variations in isoform expression leading to alterations in the electrophysiological properties of the tissue. Extreme alterations in expression in nonpreferential locations can disrupt $I_h$’s pacemaking capabilities. This disruption in the duality between functionality and channel composition has been well documented in cardiomyocytes. In a study where female guinea pig atrial myocytes overexpressed HCN1, it was observed that overexpression of HCN1 inhibited the cardiomyocyte’s ability to generate spontaneous action potentials (Lieu et al., 2008). It is worth noting the results of a study using a mathematical model that looked into the effects of altering the ratio of $I_{K1}$, which is an inwardly rectifying current that acts to stabilize the resting membrane potential, and $I_f$ on the automaticity of the cardiomyocytes (Lieu et al., 2008). There was an observed restoration in the automaticity when the ratio of $I_f$ to $I_{K1}$, was balanced. This would suggest that in some instances that there is an duality in expression between $I_f$ channel and other voltage gated channels, in this case $I_{K1}$, that needs to be met to confer the cell with its automaticity.

While the alteration of HCN isoform expression effects the physiological functioning of cardiomyocytes, the same expression dysfunction dynamic has also been observed in neurons.
HCN channels have received particular attention as a candidate for dysfunction in epileptic syndromes due to their regulatory capabilities. Over the years, numerous studies have provided evidence for the role of HCN in both acquired and genetic epilepsies. In humans, mutation analysis has revealed sequence variation in HCN1 and HCN2 associated with epilepsy (Dibbens et al., 2010, Tang et al., 2008). One of these studies observed a triple proline deletion that occurred more often in patients with febrile seizures (Dibbens et al., 2010). This mutant variant increases HCN2 channel function.

Variations in HCN1 and HCN2 expression have been well documented in animal epilepsy models. One such study observed a variation in HCN1 and HCN2 distribution in the hippocampus of epileptic rats. This is due to a downregulation in HCN1 expression and an upregulation in HCN2 expression (Brewster et al., 2002). This alteration in isoform expression has been observed in other murine models as well as in other brain regions. Altered HCN2 expression in thalamocortical and thalamic reticular neurons in HCN2-deficient mice have been observed to display a phenotype of absence epilepsy (Ludwig et al., 2003). In the developing brain, it has been observed that rising levels of HCN1 are associated with reduced dendritic excitability (Chen et al., 2001). Increased HCN1 expression in thalamocortical neurons in a rat model has also been observed in absence epilepsy (Buddle et al., 2005).

**Anti-convulsants and HCN Channels**

The mechanism of action of many of the current antiepileptic drugs (AED) used today is incompletely understood. Lamotrigine’s anticonvulsant properties are believed to work primarily by inhibiting voltage-gated sodium channels (Teoh et al., 1995). Since
the initial discovery of its interaction with voltage-dependent sodium channels, lamotrigine has been reported to modulate the activity of other voltage gated ion channels such as high voltage activated calcium channels (Pisani et al., 2004, Stefani et al., 1996, Wegerer et al. 1997, Wang et al., 1998), outward rectifying voltage dependent potassium channels (Grunze, et al., 1998, Huang et al., 2004, Zona et al., 2002), and inward rectifying HCN channels (Peng et al., 2010, Poolos et al., 2003). Lamotrigine has been shown to enhance $I_h$ in hippocampal CA1 pyramidal neurons, decrease the passive propagation of excitatory postsynaptic potentials from distal dendrites to the soma, and negatively shift the voltage-dependence of $I_h$ activation (Peng et al., 2010, Poolos et al., 2003). Lamotrigine increases $I_h$ in CA1 interneurons without altering the voltage-dependence of $I_h$ activation (Peng et al., 2010). These cells, in the presence of lamotrigine, have a more depolarized membrane resulting in an increase in inhibitory neuron firing onto the CA1 pyramidal cells. This results in an increased inhibition of these postsynaptic neurons. Furthermore, lamotrigine has been shown in vitro to decrease the duration of synchronized burst activity in CA1 through an $I_h$-mediated mechanism (Adams et al., 2009). Interestingly, lamotrigine has been reported on inhibit postsynaptic AMPA receptor and glutamate release (Philips et al., 1997, Lee, et al., 2008). Thus, lamotrigine appears to have a broader clinical profile than would be expected based on a single mechanism.

Conclusion

$I_h$ has been implicated in seizures, but its role is diverse as well as complex. Its complexity lies in the observations that different HCN types can contribute differently, that both up- or downregulation can be associated with the disease, and that the role of an
HCN channel strongly depends on its cellular localization (Biel et al, 2009). Of the channelopathies identified in epilepsy, the role of HCN channels in the epilepsy disease state is relatively unclear. In this present study, the effects of altering HCN channel activity on the intrinsic and polysynaptic network properties of cells in the 4-AP hyperexcitability model were examined.

METHODS

Slice Preparation and Electrophysiological Recordings

All experiments were performed in accordance with the National Institutes of Health Guide for the Care of Laboratory Animals using protocols approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Neocortical slices were prepared from 20- to 25-day old (PN 20-25) Sprague-Dawley rats. Rats were anesthetized with isoflurane before being decapitated. The brains were quickly removed and placed in ice cold cutting solution which contained (in mM): 135 N-methyl-D-glucamine (NMDG), 1.5 KCl, 1.5 KH2PO4, 23 choline HCO3, 0.4 ascorbic acid, 25 D-glucose, 3.5 MgCl2 and 0.5 CaCl2 (Tankaka, et al, 2008). This solution was bubbled with 95% O2/ 5% CO2 to maintain a pH 7.4. Coronal brain slices (300 μm thick) were cut from the anterior portion of the brain with a vibratome (Microm, Walldorf, Germany) and transferred to an incubation chamber for 30 to 45 minutes at 37 degree C° in a recording solution containing (in mM): 125 NaCl, 3.5 KCl, 26 NaHCO3, 10 D-Glucose, 2 MgCl2 and 2 CaCl2 which was be continuously bubbled with 95% O2/ 5% CO2 to maintain a pH around 7.4. The slices were transferred from the incubation chamber and allowed to sit at room temperature for 30 minutes before use.
Individual slices were subsequently transferred to a recording chamber perfused at 3-4 mL/min. with a recording solution that was temperature regulated between 30-32°C. Neurons for whole-cell recording were visualized using a Zeiss Axio Examiner D.1 (Carl Zeiss, Thornwood, NY) microscope equipped with Dodt contrast optics, a 40X-water immersion lens, and infrared illumination. L5 pyramidal cells were identified by their morphology and distance from the pial surface. Recording electrodes were loaded with 0.5% biocytin which was allowed to diffuse into the cell for additional intracellular labeling to confirm identification. Labeled cells were processed as described previously (Zhou and Hablitz, 1996). Whole cell voltage clamp and current clamp techniques were used with patch electrodes having open tip resistances of ~3 MΩ resistance and filled with an intracellular solution containing (in mM): 125 K-gluconate, 10 KCl, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA (pH and osmolality were adjusted to 7.4 and 290 mosM respectively). Tight seals (>1.5 GΩ) were obtained before breaking into whole-cell mode. Signals were acquired using a Warner Instruments PC-505 amplifier (Warner Instruments Corporation, Hamden, CT) controlled by Clampex 8.2 software (Molecular Devices, Inc., Sunnydale, CA) through a Digidata 1322A interface (Molecular Devices). Responses were filtered at 1 kHz and digitized at 10-20 kHz. A bipolar stimulating electrode (twisted pair of 25 μm Formvar-insulated nichrome wires) was used to provide square wave current pulses 100-400 μA in amplitude for 200 μs to evoke synaptic responses. The stimulating electrode was positioned intracortically at a distance of 100-200 μM adjacent to the recording pipette. Either 20 μM ZD7288 (4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride) or 100 μM lamotrigine were bath applied after control recordings were obtained in the presence of 50 μM 4-AP.
**Application of Pharmaceuticals**

Lamotrigine and ZD7288 were obtained from Tocris Bioscience whereas 4-AP was obtained from Sigma-Aldrich Co. (Saint Louis, MO). Frozen stock solutions of the drugs were dissolved in the recording aCSF prior to each experiment. All drugs were bath applied. Biocytin was obtained from Sigma-Aldrich Co. Biocytin was dissolved prior to each recording in an intracellular recording solution.

**Data Analysis**

Responses were analyzed offline using Clampfit 9.0 software (Molecular Devices, Inc., Sunnydale, CA). Data was analyzed using Microsoft Excel (Microsoft, Redmond, WA) and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). The duration of the epileptiform discharges were determined for each cell by comparing sweeps with equivalent membrane potentials following hyperpolarizing current injections from control and treated recordings. The duration of the sweep was measured for each cell by subtracting the time point at which the postsynaptic potentiation plateaued from the time point post-stimulus preceding the potentiation. The duration of inhibition was measured by subtracting the time point preceding the start of the action potential by the time point following the extracellular stimulation.

In response to a hyperpolarizing current pulse, a voltage response is observed in which an apex is reached after which voltage “sag” is observed due to the I_h associated inward current. The voltage sag amplitude was measured following a hyperpolarizing
current injection by subtracting the voltage at which the response plateaued from the voltage at the trough of the response.

The chloride reversal potentials were calculated for the different experimental conditions using the Nernst Equation:

\[ E_{Cl^-} = \frac{(R \times T)}{(z \times F)} \ln \frac{[C_o]}{[C_i]} \]

\( R = \) ideal gas constant = 8.314 JK\(^{-1}\)mol\(^{-1}\)
\( T = \) absolute temperature = 312.314 K
\( z = \) ion charge = -1
\( F = \) Faraday’s constant = 9.648 X 10\(^4\) Cmol\(^{-1}\)
\( C_o = \) the concentration of chloride ions outside the cell = 132.5 mM
\( C_i = \) the concentration of chloride ions inside the cell = 10 mM

Voltage clamp data were also used to calculate reversal potentials and conductance. IV-plots were generated in Graphpad Prism. Linear regression analyses were performed on the plots from which the reversal potential (x-intercept) and the conductance (slope\(^{-1}\)) were extrapolated. Data are expressed as mean ± SEM. For data expressed in percentage difference, measurements from drug treated cells were normalized to their corresponding nondrug treated cells using GraphPad Prism. Statistical analysis was carried out using two-tailed paired Student’s t-test with a 95% confidence interval.

**RESULTS**

*Characterization of the Effect of 4-AP on the Intrinsic Properties of Neocortical L5 Pyramidal Cells*

4-AP has been shown to have convulsive properties in both *in vivo* and *in vitro* (Szente and Baranyi, 1987, Chestnut and Swann, 1988, Galvan et al., 1982, Rutecki et al.,
1987, Mattia et al., 1993, Traub et al., 1995, Perreault, 1991, Buckle and Haas, 1982). It has been well documented that 4-AP can enhance synaptic transmission (Arvanov et al., 1995, Galavan et al., 1982, Buckle and Haas, 1982, Gu et al., 2004, Flores-Hernández et al., 1994) and block outward rectifying potassium currents (Perreault and Avoli, 1989, Arvanov et al., 1995, Gutmann et al., 2005, Storm, 1988). Inhibition of the transient outward A-type potassium current (I\textsubscript{A}) can result in a prolonged action potential waveform, a reduction in firing threshold, a reduced latency to first spike, and an increased interspike interval (Rudy, 1988, Schoppa and Westbrook, 1999, Schibata et al., 2000, Molineux et al., 2005, Jeng et al., 2004, Carrasquillo et al., 2012). While I\textsubscript{A} can affect excitability and synaptic integration in neurons, I\textsubscript{h} too can influence synaptic integration and excitability in neurons (Magee, 1998, Park et al., 2011, Pavlov et al., 2011, Wiershke et al., 2010, Williams and Stuart, 2000, Atherton et al., 2010), but I\textsubscript{h} can also control the resting membrane potential in neurons (Nolan et al., 2007, Bayliss et al., 1994, Mayer and Westbrook, 1983, Solomon and Nerbonne, 1993, Lamas, 1998). Thus, in this present study we wanted to examine the effects altering I\textsubscript{h} would have on the 4-AP model of epilepsy in which synaptic transmission is enhanced.

Because the effects of 4-AP on the intrinsic properties has been well documented in hippocampal neurons (Chestnut and Swann, 1998, Avoli, 1990, Perreault and Avoli, 1989), we first wanted to establish the effects of 4-AP on some of these properties without altering I\textsubscript{h} in L5 pyramidal neurons. 4-AP can block various voltage gated potassium channels depending on the concentration used (Arvanov et al., 1995, Gutmann et al., 2005, Storm, 1988). The concentration of 4-AP (50 μM) used in the present study has been observed to block I\textsubscript{A} (Storm, 1988). Somatic whole cell patch clamp technique
was utilized to obtain recordings before and after bath application of 4-AP. Slices were prepared as mentioned above from rats PN 20-25 from which current clamp recordings were be obtained from visually identified L5 neocortical pyramidal cells based on their morphology and distance from the pial surface (Fig.-1). The inward current mediated by \( I_h \) results in a depolarization of the resting membrane potential (Magee, 1998). Thus, we wanted to initially evaluate changes in the resting membrane potential resulting from exposure to 4-AP. The resting membrane potential was determined prior to the initiation of the current clamp protocols before and after bath application of 4-AP (Fig-2). No deviation in the resting membrane potential was observed following the bath application of 4-AP (control: -67.47 ± 1.189 mV; 4-AP: -67.26 ± 3.151 mV; n=5; p=0.4174; Fig.-2). \( I_h \)’s contribution to the cell’s resting membrane potential results in an increase in the cell’s membrane conductance making the input resistance an important measurement to analyze when investigating alterations in the cell’s intrinsic properties (Magee, 1998). Hyperpolarizing current steps (Fig-2) were used to examine changes in the input resistance resulting from treatment with 4-AP. Treatment with 4-AP resulted in no change in the input resistance (control: 105.1 ± 21.24 MΩ; 4-AP: 96.71 ± 16.74 MΩ, n=5; p=0.4013). Due to the combined changes in input resistance and resting membrane potential resulting from \( I_h \), it is difficult to predict the net effect on the cell’s intrinsic excitability (Dyhrfjeld-Johnson et al., 2009). Thus, we examined AP firing resulting from somatic current injections (Fig.-1). Following treatment with 4-AP, no change in the number of AP’s resulting from somatic current injections were observed (control: 29 ± 5; 4-AP: 31 ± 7; n=4; p=0.486; Fig.-1).
Hyperpolarization of the membrane potential activates HCN channels resulting in the passage of an inward current which is visible as a prominent depolarizing “sag” in the membrane potential (McCormick and Pape, 1990). This $I_h$-associated sag was visible in our recordings (Fig-2). Treatment with 4-AP resulted in no change in the sag amplitude (control: $6.449 \pm 1.952 \text{ mV}$; 4-AP: $7.432 \pm 1.829 \text{ mV}$, $n=5$; $p=0.1821$; Fig.-2).

**Formation of Epileptiform Discharges Following Treatment with 4-AP**

4-AP has been documented to increase the duration and amplitude of evoked EPSP’s (Perreault and Avoli, 1991) while $I_h$ has been observed to cause increases in the dendrosomatic attenuation of somatic EPSP amplitudes (Biel et al., 2009). $I_h$ has been observed to alter GABAergic and glutamatergic synaptic transmission in neurons of the basolateral amygdala and globus pallidus, and pyramidal cells of the subthalamic nucleus (Boyes et al, 2007, Atherton et al, 2010, Park et al, 2011). Thus, we wanted to observe the effects of altering $I_h$ in the 4-AP model of epilepsy in which synaptic transmission in enhanced. We first had to establish the effects of 4-AP on PSP’s in L5 pyramidal cells. Using the somatic whole cell patch clamp technique, polysynaptic responses were evoked with a stimulation electrode placed in L5 of the neocortex. Upon treatment with 4-AP, the evoked epileptiform discharges observed consisted of postsynaptic potentiations (PSP)’s lasting longer than 400 ms (Fig.-3). No significant change in the polysynaptic response amplitude (control: $21.56 \pm 5.41 \text{ mV}$; 4-AP: $27.75 \pm 4.91 \text{ mV}$; $n=5$; $p=0.3413$; Fig.-3) resulting from the treatment with 4-AP was observed.

It has been noted in the 4-AP hyperexcitability model that there is an increase in the duration of PSP’s (Rutecki et al., 1987, Perreault and Avoli, 1989, Traub et al., 1995).
in the hippocampus. $I_h$ has also been observed to increase the duration of PSP’s (Williams and Stuart, 2000, Williams and Stuart, 2003). Thus, we wanted to find out how altering $I_h$ would affect the duration of the PSP’s, but first needed to establish how 4-AP would affect the duration of PSP’s in L5 pyramidal cells. Evoked PSP’s in the presence of 4-AP showed a significant increase in duration (control: 0.258 ± 0.054 ms; 4-AP: 1.022 ± 0.042 ms; n=5; p=0.0003; Fig.-3).

$I_h$ has been observed to alter GABAergic and glutamatergic synaptic transmission in neurons of the basolateral amygdala and globus pallidus, and pyramidal cells of the subthalamic nucleus (Boyes et al, 2007, Atherton et al, 2010, Park et al, 2011). Under conditions where inhibitory and excitatory synaptic signaling is enhanced, we wanted to observe how long it would take during a train of action potentials for the cell to recovery from an evoked polysynaptic responses to once again start rapidly, synchronously firing while altering $I_h$. We labeled this measurement the “duration of inhibition.” Evoked polysynaptic responses resulted in a significant increase in the duration of inhibition following treatment with 4-AP (control: 0.120 ± 0.030ms; 4-AP: 0.792 ± 0.168ms; n=5; p=0.0121; Fig.-3).

*Voltage Clamp Analysis of the Effects of 4-AP on Postsynaptic Currents*

Voltage clamp was utilized to examine the effects of pharmacologically altering $I_h$ on voltage-dependent and time-dependent ionic conductance. We used voltage clamp to better examine $I_h$’s effect on synaptic activity, but we first wanted to examine polysynaptic responses generated from bath application of 4-AP. Cells were held at -70 mV and exposed to a series of command potentials steps ranging from -40 mV to -120
mV (Fig.-4). Once cells reached a steady state, polysynaptic responses were evoked using intracortical stimulations with twist wire stimulation electrode. The input resistance as well as the duration and amplitude of PCS’s were analyzed before after bath application of 4-AP. The input resistance was analyzed for the voltage clamp recordings as added assurance of continuity between experimental techniques. Once again, no significant change in input resistance was observed resulting from treatment with 4-AP (control: 88.11 ± 14.32 MΩ; 4-AP: 86.60 ± 14.97 MΩ; n=4; p=0.8923; Fig.-4).

\(I_h\) has been implicated in attenuating amplitude and duration for both IPSPs and EPSPs (Williams and Stuart, 2000, Williams and Stuart, 2003). Thus, in a model were synaptic transmission is enhanced, we wanted to examine how altering \(I_h\) would the current amplitude and duration from evoked epileptiform discharges. Following the application of 4-AP, there was no observed change in the maximum amplitude of the inward response at -60 mV or at the any of the other subsequent membrane potentials (Table-1, Fig.-4). A significant increase in the duration of epileptiform discharges was observed at the -80 mV, -100 mV, and -120 mV command potentials (Table-1) (Fig.-4).

**Intrinsic Properties of L5 neurons Following Inhibition of \(I_h\)**

\(I_h\), being activated and inwardly directings at rest, depolarizes the resting membrane potential and decreases the input resistance of the cell. The overall effect of these opposing properties of \(I_h\) on the intrinsic excitability of the cell depends on other factors such as density of \(I_h\) and other voltage-dependent channels on the dendrites (Dyhrfjeld-Johnsen et al., 2009, Noam et al., 2011). Having established the effects of 4-AP on the intrinsic properties of L5 neurons, we wanted to examine the effects \(I_h\).
inhibition would have on these neurons while in a 4-AP induced hyperexcitable network state. Whole cell patch clamp technique was utilized to obtain recordings before and after bath application of 20 μM ZD7288, an HCN channel antagonist (BoSmith et al., 1993, Harris and Constanti et al., 1995). The same step protocol utilized as previously used for the 4-AP experiments. The resting membrane potential was determined prior to the onset of the somatic current injection before and after bath application of ZD72288. Application of ZD7288 lead to a significant hyperpolarizing shift in the resting membrane potential (control: -63.53 ± 0.923 mV; ZD7288: -67.34 ± 1.41 mV; n=10; p=0.016; Fig.-6). Changes in input resistance and voltage sag amplitude were analyzed following hyperpolarizing somatic current injections. ZD7288 application resulted in an increase in the input resistance (control: 43.64 ± 1.696 MΩ, n=6; ZD7288: 55.36 ± MΩ, n=6; p=0.0172; Fig.-6) and a decrease in the voltage sag amplitude (control: 2.465 ± 0.3875 mV; ZD7288: 0.4538 ± 0.1948 mV, n=7; p=0.003; Fig.-6).

We also wanted to investigate the effects of Ih inhibition on the intrinsic firing properties. Following intracellular stimulation, regular spiking was observed in the majority of the cells with one cell producing tonic bursting. Upon bath application of ZD7288, a significant increase in the number action potentials was observed in response to a 500 pA current injection (control: 10 ± 1, n=9; ZD7288: 18 ± 4, n=9; p=0.0471; Fig.-5). From these experiments for assessing intrinsic excitability, statistically significant increases in spiking and input resistance, a significant decrease in voltage sag amplitude, and a significant hyperpolarizing shift in the resting membrane potential were observed following Ih inhibition with ZD7288.

*Alterations of Epileptiform Discharges Following Ih inhibition*
To investigate the network response resulting from inhibition of $I_h$ while in the presence of 4-AP, we initially utilized the whole cell patch technique. The protocol utilized for assessing 4-AP induced polysynaptic response changes was used again. We measured changes in the maximum amplitude between the experimental groups. No significant change in the maximum amplitude following treatment with ZD7288 (control: 46.4 ±9.89 mV; ZD7288:39.1 ± 3.54; n=7; p=0.4142) (Fig.-7). The duration of the epileptiform discharges were measured and analyzed in the same manner as the previous experiment. Treatment with ZD7288 resulted in a significant increase in the duration of the evoked epileptiform discharge (control: 0.68±0.099s; ZD7288: 1.66±0.359; n=7; p=0.0284) (Fig.-7).

We also wanted to investigate how inhibiting $I_h$ would affect the duration of inhibition. When ZD7288 was applied, there was an observed increase in the duration of inhibition (control: 0.6121 ± 0.1565; ZD7288: 1.234 ± 0.2974; n=7; p=0.0294) (Fig.-7). From the whole cell current clamp experiments during which polysynaptic responses were evoked, significant increases in the duration of inhibition and the duration of epileptiform discharge were observed following treatment with ZD7288.

Voltage Clamp Analysis of Polysynaptic Responses Following $I_h$ Inhibition

It has been well documented that 4-AP induced epileptiform discharges in the hippocampus can be made up of three components: an early EPSC, an early IPSC, and a late IPSC (Traub et al., 1995, Rutecki et al., 1987, Perreault and Avoli, 1989). Thus, we wanted to examine the effects of $I_h$ on these 3 currents. The same voltage clamp protocol as previously used was utilized. The input resistance was examined to ensure continuity.
in the observed changes in I_h between the two patch clamp techniques. Analysis of the input resistance revealed a significant increase in the input resistance resulting from the application of ZD7288 (control: 48.85 ± 1.696 MΩ; ZD7288: 55.63 ± 55.63 MΩ; n=7; p=0.0086) (Fig.-8). Intracortical stimulation resulted in a biphasic response, outward current followed by an inward directed current, at the -40mV and -60 mV command potentials (Fig.-8). At the command potentials more hyperpolarized than -60 mV, the current was outwardly directed. The duration and amplitude of the evoked PCS’s were analyzed from recordings taken before after bath application of ZD7288. After the application of ZD7288, the maximum amplitude of the inward response at -60 mV was not significantly different at all of the measured membrane potentials (Table-2) (Fig.-8). A significant increase in the duration of epileptiform discharges were observed at -80 mV, -100 mV, and -120 mV command potentials (Table-2) (Fig.-8). We also analyzed the reversal potentials to investigate possible synaptic receptors that might be affected by alterations in I_h activity. The expected chloride reversal potential under the present recording condition was -70 mV. Because of the complexity of these evoked epileptiform discharge, we examined reversal potentials for the early excitatory and late inhibitory conductance (Fig.-9). The reversal potential of the early excitatory current was measured at the nadir of the inward current of the biphasic response (Fig-9). A depolarizing shift in reversal potential of the early current was observed in 6 of the 7 cells. Analysis of the reversal potential of the early excitatory current revealed a significant average depolarizing shift in the reversal potential (control: -50.8±2.23 mV; ZD7288: -45.7±1.54mV; n=7; p=0.027). Treatment with ZD7288 caused a significant decrease in the conductance (control: 49.6±8.91 µS; ZD7288: 36.7±9.07µS; n=7;
p=0.044) (Fig.-9). The reversal potential for the late inhibitory current was measured at the start of this current (Fig.-9). The shift in the reversal potential for this current was not as consistent amongst the recordings as the early excitatory current. In 4 of the 7 recordings, a hyperpolarizing shift in the reversal potential was observed. In the other 3 of the recordings, a depolarizing shift in the reversal potential was observed (control: -50.54±2.61mV; ZD7288: -61.14±1.81mV; n=6; p=0.0617) (Fig.-9). Analysis of the conductance revealed increases in 4 cells, yet in the other 3 cells, the conductance decreased (control: 48.6±6.74μS; ZD7288: 46.3±8.39μS; n=7; p=0.78) (Fig.-9).

**Intrinsic properties of L5 neurons Following Enhancement of Ih**

In the previous experiment, we observed changes in intrinsic firing properties due to inhibition of Ih. Thus, we wanted to investigate the effects enhancing Ih would have on the intrinsic firing properties of L5 pyramidal cells. The same current clamp protocol was utilized to examine the intrinsic properties as in the previous experiments with the exception of the bath application of lamotrigine, a known Ih agonist (Adams et al., 2009, Berger and Lüscher, 2004, Poolos et al., 2003, Peng et al., 2010). Application of lamotrigine resulted in a depolarizing shift in the resting membrane potential (control: -61.2 ± 0.6464 mV, n=14; lamotrigine: -63.13 ± 0.737 mV; n=13; p=0.0003)( Fig.-11) while the input resistance was not significantly altered (control: 113.8 ± 6.263 MΩ; lamotrigine: 117.6 ± 8.209 MΩ; n=13; p=0.3909) (Fig.-11). In the presence of lamotrigine, there was a significant decrease in the number of action potentials in response to a 500 pA somatic current injection (control: 33.57 ± 1.771; lamotrigine: 20.86 ± 2.344; n=7; p=0.003) (Fig.-10). Application of lamotrigine also significantly
increased the sag amplitude (control: 6.601 ± 0.6840 mV; lamotrigine: 7.563 ± 0.5717 mV; n=11; p=0.0053) (Fig.-11).

Alteration of Epileptiform Discharges Following Enhancement of $I_h$

We also examined the effects of $I_h$ enhancement on evoked epileptiform activity. We analyzed the duration, maximum amplitude, and duration of inhibition. After bath application of lamotrigine, the maximum amplitude of the evoked postsynaptic potential decreased (control: 21.41 ± 2.579; lamotrigine: 16.15 ± 2.604; n=11; p=0.002) (Fig.-12) while the duration of the evoked epileptiform discharge was also significantly reduced (control: 0.6097 ± 0.09214; lamotrigine: 0.2551 ± 0.1646; n=11; p<0.0001) (Fig.-12). Bath application of lamotrigine also lead to a significant decrease in the duration of inhibition (control: 0.5311 ± 0.1059; lamotrigine: 0.2979 ± 0.04615; n=8; p=0.0327) (Fig.-12). Significant changes in the duration, maximum amplitude, and latency to spike in the presence of lamotrigine were observed while only changes in duration and latency to spike were observed during the bath application of ZD7288.

Voltage Clamp Analysis of Polysynaptic Responses Following $I_h$ Enhancement

After observing the changes in network excitability resulting from $I_h$ inhibition in voltage clamp, we wanted to examine how enhancing $I_h$ would affect network excitability under voltage clamp recording conditions. The same voltage clamp protocol was used as in the previous experiment with ZD7288 except lamotrigine was bath applied after control recordings were taken. As seen in the current clamp experiment with lamotrigine, the input resistance did not significantly change (control: 107 ± 11.11; lamotrigine: 115.7
The duration of epileptiform discharges was significantly decreased for all membrane potential steps (Table-3, Fig.-13). The maximum amplitude also decreased for the all of the voltage steps except for the -40 mV step (Table-3, Fig.-13). We also examined the reversal potential to investigate possible synaptic receptors that might be affected by alterations in I_h activity. The expected chloride reversal potential under the present recording condition was -70 mV. We examined reversal potentials of the early excitatory and late inhibitory currents as previously examined in the ZD7288 experiments (Fig.-14). For the early current, there were inconsistent depolarizing and hyperpolarizing shifts in the reversal potential resulting in no observable change in the early current reversal potential resulting from lamotrigine treatment (control: -53.56 ± 2.638; lamotrigine: -53.33 ± 3.066; n=6; p=0.9303) (Fig.-14). In 5 of the 6 cells treated with lamotrigine, there was a corresponding increase in the conductance (Fig.-14). Analysis of the conductance revealed a statistically significant decrease in the early excitatory conductance following treatment with lamotrigine (control: 19.3 ± 3.22nS; lamotrigine: 13.2 ± 2.00nS; n=6; p=0.0233) (Fig.-14). The late inhibitory current corresponds to a long-lasting outward synaptic current. The reversal potential was taken at the time point corresponding to the start of the late inhibitory current (Fig.-14). In 4 of the 6 cells, a depolarizing shift in the reversal potential was observed. Analysis of the reversal potential, revealed no change in the reversal potential for the late inhibitory current resulting from treatment with lamotrigine (control: -64.8±1.80 mV; lamotrigine: -64.0±2.68 mV; n=6; p=0.775) (Fig.-14). In 5 of the 6 cells treated with lamotrigine, the conductance of the late inhibitory conductance decreased following treatment. Analysis of the conductance
revealed treatment with lamotrigine to not have a significant effect on the late inhibitory conductance (control: 21.8 ± 3.08nS; lamotrigine: 17.8±3.03; n=6; p=0.1051) (Fig.-14).
Figure 1: The effect of 4-AP on the intrinsic firing property of L5 pyramidal cells. A: Photomicrograph showing a layer 5 pyramidal cell. B and C: Specimen records in response to a depolarizing current pulse given under control conditions and after bath application of 4-AP, respectively. D: Summary of results from four cells showing number of action potentials evoked before (■) and after (●) bath application of 4-AP. The outer data point represents the average number of action potentials with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. No significant differences were observed.
Figure 2: The effects of 4-AP on intrinsic excitability and $I_h$. A and B: Specimen records in response to hyperpolarizing current pulses given under control conditions and after bath application of 4-AP, respectively. Note the presence of the $I_h$ associated “sag” denoted with (→), in both control and 4-AP recordings. C: Summary of resting membrane potential measurement from current clamp recordings before (■) and after (●) bath application of 4-AP. No significant change in membrane potential was observed. D and E: Plots depicting the effects on the input resistance and amplitude of sag response, respectively. Changes in the input resistance and sag amplitude were not significant different.
Figure 3: 4-AP alters the duration of inhibition and duration of epileptiform discharges. A and B: Specimen records of evoked epileptiform discharges at a hyperpolarized current step under control conditions and after bath application of 4-AP, respectively. C and D: Graphical representations of the effect of 4-AP on the maximum amplitudes and the duration of epileptiform discharges before (■) and after (●) bath application of 4-AP. 4-AP significantly increased the duration of epileptiform discharges, but did not affect the maximum amplitudes. E and F: Specimen records of the duration of inhibition under control conditions and after bath application of 4-AP, respectively. → denotes the point at which the extracellular stimulation occurred. G: Graphical representation of the duration of inhibition. 4-AP treatment resulted in an increase in the duration of inhibition. Student’s t-test *p<0.05, **p<0.01.
Figure 4: Voltage clamp analysis of polysynaptic responses following 4-AP treatment. A and B: Voltage clamp recordings of epileptiform discharges at different holding potentials before (A) and after (B) application of 4-AP. C: Graphical depiction of input resistance before (■) and after (●) bath application of 4-AP. Displayed in this graph are the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. D: Graphical depiction of the durations (in seconds) of the polysynaptic responses at the -60 mV, -80 mV, 100 mV and -120 mV command potentials. The averages are displayed with SEM error bars. Significant increases in the duration of the polysynaptic responses were observed at all of the command potentials. E: Graphical representation of the maximum current amplitudes resulting from evoked polysynaptic responses at the various command potentials. The averages are displayed with SEM error bars. 4-AP treatment did not alter the maximum amplitude. Student’s t-test *p<0.05, **p<0.01, ***p<0.001.
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</table>

Table 1: Tabulation of the maximum amplitude and duration of epileptiform discharge averages with SEM error bars at the various command potentials. # denotes an n value of 3.
Figure 5: The effect of $I_h$ inhibition on the intrinsic firing property of L5 pyramidal cells. A and B: Specimen records in response to a depolarizing current pulse given under control conditions and after bath application of ZD7288, respectively.  C: Graphical summary of the number of action potentials induced before (■) and after (▲) bath application of ZD7288. The outer data point represents the average number of action potentials with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. Treatment with ZD7288 resulted in a significant increase in the number of action potentials fired.
Figure 6: The effect of $I_h$ inhibition on the intrinsic firing properties and $I_h$ in L5 pyramidal cells. A and B: Specimen records in response to hyperpolarizing current injections under control conditions as well as following bath application of ZD7288, respectively. Note the presence of the $I_h$ associated “sag” denoted with (→), in the control recording and the lack thereof in the ZD7288 recording. D: Graphical summary of resting membrane potential data collected before (■) and after (▲) bath application of ZD7288. The outer data point represents the average resting membrane potential with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. A significant hyperpolarizing shift in the resting membrane potential was observed following treatment with ZD7288. D and E: Plots depicting the effects on the input resistance and sag amplitude, respectively. The outer data point represents the average number of action potentials with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. A significant increase the input resistance and significant decrease in the sag amplitude were observed following treatment with ZD7288. Student’s t-test *$p<0.05$, **$p<0.01$. 
Figure 7: Inhibition of $I_h$ leads to alterations in the duration of inhibition and duration of the epileptiform events. A and B: Specimen records of evoked epileptiform discharges at a hyperpolarized current step under control conditions and following treatment with ZD7288, respectively. C and D: Graphical representations of the effect of ZD7288 on the maximum amplitude and the duration of the epileptiform discharge before (■) and after (▲) bath application of ZD7288. ZD7288 significantly increased the duration of the epileptiform discharge, but did not affect the maximum amplitude. E and F: Specimen records of the duration of inhibition under control conditions and after bath application of ZD7288, respectively. → denotes the point at which the extracellular stimulation occurred. G: Graphical representation of the duration of inhibition. $I_h$ inhibition with ZD7288 resulted in an increase in the duration of inhibition. Student’s t-test *p<0.05, **p<0.01.
Figure 8: Voltage clamp analysis of polysynaptic responses following I_h inhibition. A and B: Voltage clamp recordings of epileptiform discharges at different holding potentials before (A) and after (B) application of ZD7288. Hyperpolarizing steps were made at -20 mV intervals starting with an initial command potential of -60 mV. C and D: A magnified, cropped portion of the traces from A and B respectively at the -60 mV command potential. Note the biphasic shape of the waveforms. E: Graphical depiction of input resistance before (■) and after (▲) bath application of ZD7288. Displayed in this graph are the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. F: Graphical depiction of the durations (in seconds) of the polysynaptic responses at the -60 mV, -80 mV, 100 mV and -120 mV command potentials. The averages are displayed with SEM error bars. Significant increases in the duration of the polysynaptic responses were observed at all of the command potentials. H: Graphical representation of the maximum current amplitudes resulting from evoked polysynaptic responses at the various command potentials. The averages are displayed with SEM error bars. ZD7288 treatment did not alter the maximum amplitude. Student’s t-test *p<0.05, **p<0.01.
Table 2: Tabulation of the maximum amplitude and duration of the epileptiform discharge averages with SEM at the various command potentials.

<table>
<thead>
<tr>
<th>Membrane Potential</th>
<th>-60 mV</th>
<th>-80 mV</th>
<th>-100 mV</th>
<th>-120 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum Inward</td>
<td>-753.5±203.6</td>
<td>-1700±388.1</td>
<td>-2012±377.7</td>
<td>-2187±386.8</td>
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<tr>
<td>Current Amplitude</td>
<td>(pA)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>p-values (n=7)</td>
<td>0.073</td>
<td>0.1442</td>
<td>0.4751</td>
<td>0.3728</td>
</tr>
<tr>
<td><strong>ZD7288</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Maximum Inward</td>
<td>-502.2±75.45</td>
<td>-1333±169.6</td>
<td>-2033±261</td>
<td>-2358±325.3</td>
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<tr>
<td>Current Amplitude</td>
<td>(pA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-values (n=7)</td>
<td>0.4751</td>
<td>0.3728</td>
<td>0.07469</td>
<td>0.07469</td>
</tr>
</tbody>
</table>

**Control** Epileptiform Duration:
-60 mV: 0.4792±0.1116
-80 mV: 0.5544±0.05558
-100 mV: 0.6541±0.06361
-120 mV: 0.8142±0.07469

**ZD7288** Epileptiform Duration:
-60 mV: 0.5902±0.05489
-80 mV: 0.7951±0.07551
-100 mV: 0.9208±0.05167
-120 mV: 0.9577±0.09675

p-values (n=7):
Control Epileptiform Duration:
-60 mV: 0.152
-80 mV: 0.0014
-100 mV: 0.0066
-120 mV: 0.0099
Figure 9: Voltage clamp analysis of the early excitatory and late inhibitory currents following $I_h$ inhibition. A and B: Voltage clamp recordings of epileptiform discharges at different holding potentials before (A) and after (B) application of ZD7288. The points at which early excitatory current (---) and the late inhibitory current (-----) are denoted in the recordings. C: Graphical depiction of the average current vs. membrane potential data under control (■) and ZD7288 (▲) treatment conditions for the early excitatory current with SEM error bars. D: Early excitatory current reversal potential graphically represented with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. Treatment with ZD7288 resulted in a statistically significant depolarizing shift in the reversal potential. E: The early excitatory conductance graphically depicted before and after treatment with ZD7288 with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. F: Graphical representation of the cumulative IV-plots for both control and ZD7288 recordings for the late inhibitory current. The average current is represented at the -60 mV, -80 mV, 100 mV and -120 mV command potentials with SEM bars. G: Graphical representation of the reversal potential for the late inhibitory current under control and ZD7288 applied conditions. Late inhibitory current reversal potential is graphically represented with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. No significant shift in the reversal potential for the late inhibitory current was found. H: The late inhibitory conductance graphically depicted with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. Treatment with ZD7288 did not significantly alter the cellular membrane conductance. Student’s t-test *p<0.05, **p<0.01, ***p<0.001.
Figure 10: The effect of $I_h$ enhancement on the intrinsic firing property in L5 pyramidal cells. A and B: Specimen records in response to a depolarizing current pulse given under control conditions and after bath application of lamotrigine, respectively. C: Summary of results of the number action potentials induced by a depolarizing current pulse before (■) and after (♦) bath application of lamotrigine. The outer data point represents the average number of action potentials with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. Enhancement of $I_h$ with lamotrigine resulted in a significant decrease in the number of action potentials induced. Student’s t-test **p<0.01.
Figure 11: The effect of $I_h$ enhancement on the intrinsic firing properties and $I_h$ in L5 pyramidal cells. A and B: Specimen records in response to hyperpolarizing current injections under control conditions as well as following bath application of lamotrigine, respectively. Note the presence of the $I_h$ associated “sag” denoted with (→), in the control and lamotrigine treated recordings. D: Graphical summary of resting membrane potential data collected before (■) and after (♦) bath application of lamotrigine. The outer data point represents the average resting membrane potential with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. A significant depolarizing shift in the resting membrane potential was observed following treatment with lamotrigine. D and E: Plots depicting the effects on the input resistance and sag amplitude, respectively. The outer data point represents the average number of action potentials with SEM error bars for their respective groups while the inner data points represent the individual measurements taken from each cell. A significant increase in the sag amplitude was observed following treatment with lamotrigine. Lamotrigine did not have a significant effect on the input resistance. Student’s t-test **p<0.01, ***p<0.001.
Figure 12: Enhancement of $I_h$ leads to alterations in network activity. A and B: Specimen records of evoked epileptiform discharges at a hyperpolarized current step under control conditions and following treatment with lamotrigine, respectively. C and D: Graphical representations of the effect of lamotrigine on the maximum amplitude and the duration of epileptiform discharges before ($\blacksquare$) and after ($\blacklozenge$) bath application of lamotrigine. Lamotrigine significantly decreased the duration of epileptiform discharges and the maximum amplitude of the epileptiform waveform. E and F: Specimen records of the duration of inhibition under control conditions and after bath application of lamotrigine, respectively. $\rightarrow$ denotes the point at which the extracellular stimulation occurred. G: Graphical representation of the duration of inhibition. $I_h$ enhancement with lamotrigine resulted in a statistically significant decrease in the duration of inhibition. Student’s t-test *$p<0.05$, **$p<0.01$. 
Figure 13: Voltage clamp analysis of polysynaptic responses following $I_h$ enhancement. A and B: Voltage clamp recordings of epileptiform discharges at different holding potentials before (A) and after (B) application of lamotrigine. C: Graphical depiction of input resistance before (□) and after (▲) bath application of lamotrigine. Displayed in this graph are the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. D: Graphical depiction of the durations (in seconds) of the polysynaptic responses at the -60 mV, -80 mV, 100 mV and -120 mV command potentials. The averages are displayed with SEM error bars. A significant decrease in the duration of the polysynaptic responses was observed at all of the command potentials. E: Graphical representation of the maximum current amplitudes resulting from evoked polysynaptic responses at the various command potentials. The averages are displayed with SEM error bars. Lamotrigine treatment resulted in a significant decrease in the current amplitude. Student’s t-test *p<0.05, **p<0.01.
<table>
<thead>
<tr>
<th>Membrane Potential</th>
<th>-60 mV</th>
<th>-80 mV</th>
<th>-100 mV</th>
<th>-120 mV</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maximum Inward Current Amplitude (pA)</td>
<td>-206.8±53.71</td>
<td>-450.6±98.55</td>
<td>-675.6±141.0</td>
<td>-816.7±167.0</td>
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<tr>
<td>p-values (n=9)</td>
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<td>0.0144</td>
<td>0.0031</td>
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<td><strong>Lamotrigine</strong></td>
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<tr>
<td>Maximum Inward Current Amplitude (pA)</td>
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<td>-235.6±47.66</td>
<td>-429.4±90.68</td>
<td>-497.4±111.4</td>
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<td>p-values (n=9)</td>
<td>0.0129</td>
<td>0.0005</td>
<td>0.039</td>
<td>0.0027</td>
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<tr>
<td><strong>Control</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Epileptiform Duration (s)</td>
<td>0.6669±0.1021</td>
<td>0.4081±0.05219</td>
<td>0.4768±0.0668</td>
<td>0.6431±0.1071</td>
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<tr>
<td>p-values (n=9)</td>
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<tr>
<td><strong>Lamotrigine</strong></td>
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<tr>
<td>Epileptiform Duration (s)</td>
<td>0.3675±0.0661</td>
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<td>p-values (n=9)</td>
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</table>

Table 3: Tabulation of the maximum amplitude and duration of the epileptiform discharge averages with SEM at the utilized command potentials.
A: Control

B: Lamotrigine

C: Membrane Potential (mV) vs. Current (pA)

D: Reversal Potential vs. Current (pA)

E: Conductance (nS) vs. Control vs. Lamotrigine

F: Membrane Potential (mV) vs. Current (pA)

G: Reversal Potential vs. Control vs. Lamotrigine

H: Conductance (nS) vs. Control vs. Lamotrigine
Figure 14: Voltage clamp analysis of the early excitatory and late inhibitory currents following Ih enhancement. A and B: Voltage clamp recordings of epileptiform discharges at different holding potentials before (A) and after (B) application of lamotrigine. The points at which early excitatory current (——) and the late inhibitory current (-----) are denoted in the recordings. C: Graphical depiction of the average current vs. membrane potential data under control (■) and lamotrigine (♦) treatment conditions for the early excitatory current with SEM error bars. D: Early excitatory current reversal potential graphically represented with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. Treatment with lamotrigine did not result in a shift in the reversal potential. E: The early excitatory conductance graphically depicted before and after treatment with lamotrigine with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. Treatment with lamotrigine resulted in a significant decrease the cellular membrane conductance. F: Graphical representation of the cumulative IV-plots for both control and lamotrigine recordings for the late inhibitory current. The average current is represented at the -60 mV, -80 mV, 100 mV and -120 mV command potentials with SEM bars. G: Graphical representation of the reversal potential for the late inhibitory current under control and lamotrigine applied conditions. The late inhibitory current reversal potential is graphically represented with the individual measurements taken from each cell (inner columns) and the average (outer columns) with SEM error bars. No significant shift in the reversal potential for the late inhibitory current was found. H: The late inhibitory conductance graphically depicted with the individual measurements taken from each cell (inner columns) and the
average (outer columns) with SEM error bars. Treatment with lamotrigine did not significantly alter the cellular membrane conductance. Student’s t-test *p<0.05, **p<0.01, ***p<0.001.
Discussion

In the present study, we observed increases in the duration of evoked postsynaptic potentiations in a 4-AP induced hyperexcitable environment. These responses were greater than 400ms in duration and deemed “epileptiform discharges.” Inhibition of Ih using ZD7288 further increased the epileptiform the duration of epileptiform discharges as well as increased intrinsic excitability of the cells. The increase in the duration of epileptiform discharges induced by 4-AP was attenuated through the enhancement of Ih with lamotrigine. Thus, Ih plays a role in modulating network excitability in the 4-AP model.

The inherited forms of epilepsy represents a small fraction of the overall epilepsy disorders and with the majority of genetic defects identified being related to either an ion channel or related to one of their modulatory subunits (Catterall et al., 2008). It has been well documented that downregulation or loss of HCN channels, due to genetic deletion or insult to the CNS, produces a neuronal hyperexcitability that contributes to epileptogenesis (Ludwig et al., 2003, Shah et al., 2004, Zhang et al., 2006, Huang et al., 2009, Wierschke et al., 2010). It has been observed in acute pharmacological epilepsy models that downregulation of Ih contributes to increases in neuronal excitability leading to an epileptogenic state (Jung et al., 2010, Xiangdon Chen, 2009).

In the current study, the whole cell patch clamp technique was used to assess the effects of altering Ih activity on the intrinsic firing properties and network activity in the acute 4-AP hyperexcitability model. When Ih activity was inhibited using ZD7288, we observed a hyperpolarization of the resting membrane potential, a decrease in the voltage sag amplitude, and increases in the input resistance. An increase in AP spike frequency
was also observed in response to a depolarizing current pulse. The duration of epileptiform discharges and the duration of inhibition both increased. Similar results were obtained under voltage clamp conditions suggesting that increases in epileptiform discharges were due to increased network excitability. Experiments were also performed in which I_h was enhanced using lamotrigine. Alterations in the intrinsic properties included depolarization of the resting membrane potential, an increase in the voltage sag amplitude, and an increase in the AP firing rate. Changes in the network properties included decreases in the duration of epileptiform discharges, duration of inhibition, and maximum amplitude of epileptiform events. Similar results were obtained under voltage clamp conditions suggesting that decreases in analysis duplicated the findings of decreases in the maximum amplitude and duration of epileptiform discharges.

*Effects of I_h on intrinsic excitability*

Alterations in the passive membrane properties of the cell can produce correlating alterations in the excitability of the cell. These alterations can also lead to a cellular hyperexcitability that can be observed in neurological diseases such as epilepsy. Intrinsic firing properties of neurons are speculated to be linked to epileptogenicity of brain tissue (Gutnick et al., 1982, Sanabria et al., 2001). In the present study, the effects of altering I_h activity on the intrinsic excitability of L5 pyramidal cells were investigated in a 4-AP hyperexcitability model.

I_h is an inward directing mixed cationic current that is active at rest and carried by primarily by Na^+. Because its activity at the resting membrane potential and its Na^+ conductance, I_h has a net depolarizing effect on the resting membrane potential.
During the current study, there was a hyperpolarizing shift in the membrane potential when $I_h$ was inhibited with ZD7288. This observation has been documented in vitro in CA1 pyramidal cells (George et al., 2009) as well as in L5 pyramidal cells (Albertson et al., 2011). Conversely, enhancement of $I_h$ resulted in a depolarizing shift in the resting membrane potential. $I_h$’s activity at the resting membrane potential also means that it will contribute toward lowering the membrane resistance (Ludwig et al., 2003, Nolan et al., 2004). Thus, inhibition of $I_h$ causes an increase in the input resistance (Albertson et al., 2011, George et al., 2009, Lewis, et al., 2011, Rosenkranz, 2011). Similar results were observed in the present study under during voltage clamp and current clamp recordings. Interestingly, this effect was not observed when $I_h$ was enhanced with lamotrigine which could be due to a secondary effect of this drug. It has been observed that both lamotrigine and ZD7288 inhibits voltage gated Ca$^{2+}$ channels (Pisani et al., 2004, Stefani et al., 1996, Sánchez-Alonso et al., 2008) but the inhibitory effects of ZD7288 on T-type Ca$^{2+}$ were reported at 100μM, which is well outside the concentration in the present study. Lamotrigine’s primary anticonvulsant effect was believed to be through inhibition of voltage-dependent sodium channels (Gunze et al., 1998, Leach and Miller, 1986, Xi et al., 1995). Since this discovery, lamotrigine has been reported to effect other ion channels including inhibition of voltage gated calcium channels that mediate P/Q- and N-type currents (Pisani, 2004), enhancement of HCN channels (Poolos, 2002), and inhibition of outward rectifying potassium channels that carry $I_D$ (Grunze, 1998) and $I_A$ (Huang, 2004). Alterations in these conductances should not result in an increase in the input resistance because, with the exception of $I_h$, all of these currents are activated at membrane potentials that are
depolarized with respect to the resting membrane potential so that they would not be active when the input resistance was determined (Gutman et al., 2005, Catterall et al., 2005).

L5 pyramidal cells are subdivided based on their observed firing patterns (Connors, 1982, McCormick, 1985, Crotchet and Petersen, 2009). These observed firing patterns are regular spiking and burst firing with regular spiking cells making up the majority of the population of these cells. For L5 pyramidal cells, action potential initiation occurs in the distal initial segment (Palmer and Stuart, 2006), thus the intrinsic excitability of the somatic membrane plays an important role in the induction of action potentials. When we tested the intrinsic firing properties through current induced action potentials, we observed an increase in the number of action potentials in the presence of ZD7288 while observing a converse decrease in the number of action potentials while lamotrigine was in the bath. The increase in intrinsic firing when \( I_h \) is inhibited has been attributed to the increase in input resistance that accompanies the hyperpolarizing shift in the resting membrane potential. In granule neurons, application of ZD7288 resulted in an increase in action potential firing (Chen, 2004). ZD7288 has also been reported to increase the intrinsic firing rate of L5 pyramidal cells in the presence of bicuculline methiodide (Albertson, 2011). The converse results have also been observed with lamotrigine (Kuo et al, 1997, Poolos et al., 2003, Xie et al., 1995). Lamotrigine has been reported to decrease the AP firing rate in CA3 hippocampal neurons resulting from somatic and dendritic current injections (Poolos et al., 2003).

*Effects of \( I_h \) on polysynaptic network excitability*
The neocortical network is comprised of excitatory pyramidal neurons and inhibitory interneurons which are laminated into six layers (Douglas and Martin, 2004, Lewis, 1878). Neurons amongst these layers are endowed with differing respective morphologies and unique physiological properties. The neocortex is divided up into circuits through which neurons synaptically integrate afferent signals which can lead to the transmission of inter- and intra-laminar efferent signals. The neocortex is responsible brain functions which include memory, language, creativity, perception, cognition, emotion, and the synthesis of movement. L5 neocortical neurons primarily connect the cerebral cortex with subcortical regions. L5 gives rise to all of the principal cortical efferent projections to subcortical structures such as the basal ganglia, brainstem and spinal cord. Neocortical L5 has been identified as the site of origin of interictal discharges in adult rats from neocortical brain-slice models of acute chemically-induced and post-traumatic epileptogenesis.

Another unique property of neurons in this layer is the distribution of HCN channels along the somatodendritic membrane. In hippocampal and cortical pyramidal cells, histological analysis has revealed a 6-fold increase in the density of HCN channels from the soma out to the dendrites (Magee, 1998, Williams, 2000, Lőrinicz, 2002). With Ih increasing the decay time of incoming EPSP’s and the distribution of HCN channels, Ih filters afferent signals resulting in attenuated EPSP’s. Thus, we wanted to observe the effects enhancing and inhibiting Ih would have on polysynaptic network activity in the 4-AP hyperexcitability model. We observed increases in the duration of evoked epileptiform responses when Ih was inhibited as well as decreases in the duration of epileptiform discharges when Ih was enhanced. This finding demonstrates that Ih plays a
role in the alteration of the duration of evoked PSP’s observed in the 4-AP hyperexcitability model.

4-AP is known to prolong action potentials and enhance synaptic potentials (Rutecki et al., 1987, Perreault and Avoli, 1989). 4-AP has been shown to induce evokeable GABA mediated long-lasting depolarizations (LLD)s (Perreault and Avoli, 1989; Perreault and Avoli, 1992). Ih can attenuate somatic EPSP’s by decreasing the decay time of dendritic EPSPs which can result in a decrease in temporal summation (Magee, 1998, Berger et al., 2001). Thus, inhibiting Ih can lead to a larger somatic EPSP. In the present study, it was hypothesized with the overall EPSP enhancement, due to 4-AP and Ih inhibition, that there should be a resulting increase in amplitude and duration of induced epileptiform activity. This, however, was only partly observed. In current clamp and voltage clamp recordings, there was an observed increase in the duration of epileptiform discharges while no observable change was found in the amplitude. Conversely, with the enhancement of Ih, there was an observed decrease in duration and amplitude of epileptiform discharges. The lack of change in amplitude due to inhibition of Ih with ZD7288 may be due to conflicting effects of 4-AP and ZD7288. ZD7288 has been reported to inhibit postsynaptic glutamate receptor mediated responses in hippocampal dentate granule cells (Chen, 2004). Perhaps, even though the time period which neurotransmitter vesicle release has increased, the number of receptors being activated is decreased. Since diminished Ih would lead to a decrease in dendritic filtering, the overall duration of the postsynaptic potentiation would increase. However, this would be scenario in which the amplitude of the postsynaptic potentiation observed at the soma would be decreased.
When discussing the effects on HCN and polysynaptic activity, the reports of presynaptic HCN channels must be considered since this could also be a potential mechanism through which the observed somatic potentiations are affected. Mammalian presynaptic $I_h$ in the central nervous system was first observed in mouse cerebellar basket cells (Southan et al., 2000). Since then, presynaptic $I_h$ has been observed in areas of the mammalian brain which include the basolateral amygdala (Park et al., 2011), brainstem (Cuttic et al., 2001), entorhinal cortex (Cunningham and Jones, 2000), globus pallidus (Boyes et al., 2007), and hippocampus (Lupica et al., 2001, Notomi and Shigemoto, 2004, Aponte et al., 2006, Bender et al., 2007, Tokay et al., 2009, Peng et al., 2011). In glutamatergic neurons, $I_h$ has been observed to decrease neurotransmitter release in rodent hippocampal, entorhinal cortical brain regions (Cunningham et al., 2004, Tokay et al., 2009, Huang et al., 2011). The effects of $I_h$ on GABA-ergic interneurons seems to be more contrasting based on the anatomical location of the interneurons within the central nervous system. Observations of an inverse correlative relationship between $I_h$ and GABA release have been observed in the cerebellum (Southan et al., 2000) and globus pallidus. Conversely, a direct relationship between $I_h$ and GABA release has also been observed in the hippocampus (Lupica et al., 2001, Aponte et al., 2006, Peng et al., 2011), the basolateral amygdala (Park et al., 2011), and entorhinal cortex (Cunningham and Jones, 2000). In mature L5 neurons, depolarizing GABA responses have been reported following dendritic GABA$_A$ activation (Gulledge and Stuart, 2003, Marty and Llano, 2005). Thus, increases in GABA release could result in an enhancement of the evoked epileptiform discharges.
In this study, we revealed in the 4-AP hyperexcitability model that altering I_h can alter network activity at the cellular and network levels. At the cellular level, alteration of I_h lead to changes in the membrane conductance and action potential firing rate. These alterations would ultimately affect how these excitatory pyramidal neurons integrate and generate signals for cell-to-cell communication. Analysis of the reversal potential of the evoked epileptiform discharges reveals that these discharges are likely GABA mediated. Voltage clamp and current clamp recordings revealed that altering I_h affected the duration of evoked epileptiform discharges. Thus, it seems that alteration of I_h in the 4-AP hyperexcitability model affects the GABAergic inputs as well as the processing of these signals in L5 pyramidal cells. Varying I_h also affected the manner in which these cells during the course of firing action potentials recovered from a local polysynaptic event to be able to start firing again. This I_h associated change in the duration of inhibition could result from changes in the dendritic conductance and/or changes at the synaptic level.

Conclusion

Epilepsy is a neurological disease that is characterized by the prevalence of chronic seizures which are caused by hyperexcitability either at the cellular level, network level, or even a combination of both. In this study, we used a 4-AP hyperexcitability model to examine the influence of I_h on network excitability. We found that inhibition of I_h lead to an increase in the intrinsic excitability of the cell. Inhibition of I_h also resulted in increases in the duration of inhibition and the duration of epileptiform discharges. Using voltage clamp recordings, we found that inhibition of I_h shifted the reversal potential of the early excitatory current in a depolarizing manner while also decreasing the cellular membrane conductance. Enhancing I_h lead to changes
in some of the intrinsic properties of the cell resulting in a net overall decrease in the intrinsic excitability. Enhancement of I\textsubscript{h} resulted in decreases in all three metrics used to assess changes in network excitability. In voltage clamp, I\textsubscript{h} enhancement resulted in decreases in the cellular membrane conductance of the early excitatory current. Enhancing I\textsubscript{h} did not have a clear effect on the late inhibitory current. Using voltage clamp and current clamp recordings, we found alterations in I\textsubscript{h} affected both network and intrinsic cellular excitability. To better elucidate the mechanism through which I\textsubscript{h} alters the duration of epileptiform discharges, experiments using pharmacological inhibition of the NMDA, GABA\textsubscript{A}, and GABA\textsubscript{B} receptors would need to be performed.
List of References


