THE ROLE OF SUBTHRESHOLD VOLTAGE-GATED POTASSIUM CHANNEL CURRENTS
IN THE DEVELOPMENT OF NEUROLOGICAL DISORDERS

A Thesis in
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by
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ABSTRACT

Voltage-gated potassium channels are critical in maintaining proper neuron signaling and excitability in the mammalian CNS. While there are a variety of diverse types of K⁺ currents, this thesis focuses specifically on subthreshold currents in the mammalian hippocampus and how they can affect the development of neurological disorders. The hippocampal region is particularly important not only in learning and memory formation but as a hot spot for the study of neurological diseases such as epilepsies and schizophrenia.

Studies regarding the roles of the subthreshold voltage-gated K⁺ currents, including the A-type and M-type currents, have elucidated new relationships between channel mutations and epilepsies. Disruptions in the M-current have already been identified in the pathogenesis of benign familial neonatal seizures while the A-current is potentially involved in the development of temporal lobe epilepsy. Further connections can be made that implicate the M-current in other neurological disorders, particularly schizophrenia.

The Kv7 channels that produce the widely studied, specialized M-current have already led to the discovery of the drug retigabine. This selective channel opener has been useful in past research regarding the role of the M-current in idiopathic epilepsies. However, retigabine and several Kv7 channel blockers may also be important in future studies that endeavor to understand how the M-current is involved in schizophrenia. Using the currently available literature, this thesis seeks to expand upon our current knowledge of the subthreshold A- and M-currents as they pertain to the pathophysiologies of neurological disorders.
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<tr>
<td>Kv</td>
<td>Voltage-gated K⁺ channel</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>AP</td>
<td>Action potential</td>
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<td>Iₐ</td>
<td>A-current</td>
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<td>Iₘ</td>
<td>M-current</td>
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<td>CA</td>
<td>Cornu Ammonis</td>
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<td>DG</td>
<td>Dentate gyrus</td>
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<td>NT</td>
<td>Neurotransmitter</td>
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<td>LTP</td>
<td>Long-term potentiation</td>
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<td>LTD</td>
<td>Long-term depression</td>
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<td>TLE</td>
<td>Temporal lobe epilepsy</td>
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<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
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<td>BFNC</td>
<td>Benign familial neonatal convulsions</td>
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<td>VTA</td>
<td>Ventral tegmental area</td>
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<td>NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
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<td>AMPAR</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor</td>
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<td>ERK</td>
<td>Extracellular signal-related kinase</td>
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<td>PKA</td>
<td>Protein kinase A</td>
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<td>PKC</td>
<td>Protein kinase C</td>
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<td>KO</td>
<td>Knockout</td>
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<td>ADP</td>
<td>Afterdepolarization</td>
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<td>Iₙₚ</td>
<td>Persistent Na⁺ current</td>
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<td>EEG</td>
<td>Electroencephalogram</td>
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<td>MEG</td>
<td>Magnetoencephalogram</td>
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Chapter 1
Introduction.

Voltage-gated K⁺ channels are important regulators of neuronal excitability in the mammalian central nervous system.

Over the course of evolution, ion channels with the K⁺-selective pore have undergone many gene duplication events resulting in a wide variety of specialized and functionally unique channel families that can exert influence over the intrinsic properties of neurons [1-3]. Voltage-gated K⁺ (Kv) channels represent large and crucial group of these potassium channels in which channel activity is regulated by the membrane voltages of neurons [1-3]. The currents that are generated by these Kv channels are especially important regulators of neuronal excitability [1-3]. There are a number of functionally diverse Kv channels, differential gene expression patterns of which are seen in many types of neurons within the complex mammalian central nervous system (CNS) [1-3]. As such, the combination of channels must be tailored to each specific neuron type to ensure normal signaling in the brain and, what’s more, dynamic regulation of the currents that they produce must be available for the fine-tuning of neuron excitability in different behavioral states [1-3].

The major structural features of the Kv channel, the K⁺ selective pore and voltage sensor domain, are illustrated in figure 1-1. Each six-transmembrane (S1-S6) channel consists of four subunits which combine to form a channel containing a two-transmembrane K⁺-selective pore that is connected to the voltage sensor domain (VSD) via a highly conserved S4-S5 linker region [1,2]. The VSD is comprised of the first four transmembrane segments (S1-S4), with S4 containing four to six charged arginine residues that function by sensing changes in membrane potential [1,2]. The linker is covalently connected to the S5 region while its residue side-chains associate with the S6 via non-covalent interactions. Therefore, any conformational changes triggered in S4 when changes in voltage are detected will cause the linker to pull upon the pore,
resulting in the outward movement of the S6 and pore opening [2]. Similarly, the linkage of the VSD to the pore is also used to prompt pore closing [2].

**Figure 1-1: Structure of the 6TM voltage-gated K+ channel.** The intracellular N-terminal region of the channel is followed by the VSD consisting of S1-S4, which is linked to the S5-S6 K⁺-selective pore, followed by an intracellular C-terminal region.

All channel families encode subunits with unique intrinsic voltage sensitivities determined by the subunit’s voltage sensor [2]. Because the intrinsic voltage sensitivity of a channel determines the membrane potential at which channel activation and deactivation occur, all channels are not open at the same voltages [2]. The implication of this is that the voltage range of activation heavily influences the functional characteristics of the current produced by a given channel.

The central focus of this thesis will be related to Kv channels that generate currents in the subthreshold range. These currents are activated at voltages below those at which action potential (AP) spikes are generated and can play important parts in the regulation of neuronal excitability at subthreshold potentials [1,2]. However, the functional specificities of these currents are far more complex than can be described by simply their activation ranges as they
are also influenced by a variety of other factors including channel kinetics, subcellular localizations, the presence or lack of inactivation, and their abilities to be modulated by receptors and chemical compounds [2]. Specifically, this thesis will explore two of the major subthreshold currents that are found in the hippocampal formation, the A-current ($I_A$) and the M-current ($I_M$).

**The hippocampal formation is an important part of the mammalian CNS.**

The hippocampus of the mammalian brain is a complex structure that can be divided up into several zones. The *Cornu Ammonis* (CA), consisting of subfields CA1 through CA4, and the dentate gyrus (DG) are the major fields of the hippocampal formation [4,5]. Although this thesis will largely focus on the CA1 and CA3, the hippocampal region in its entirety has been described as important in the regulation of learning, memory, and cognitive function [4,5].

Regulation of these functions is largely mediated by synaptic plasticity, the process by which synaptic strength is altered over time [6-8]. As the release of neurotransmitter (NT) molecules at synapses is a primary means of neural communication, it is particularly important that they can be dynamically regulated in order to maintain proper hippocampal function. Synaptic plasticity is typically carried out by the induction of either long-term potentiation (LTP) or long-term depression (LTD). LTP is the process by which the signaling at a synapse is temporally strengthened whereas LTD describes the temporal weakening of synaptic signaling and there is evidence to suggest that many ion channel currents can affect a neuron’s ability to induce either LTP or LTD, including subthreshold $K^+$ currents [6-8].

In the CA1-CA3 areas, LTP is mainly modulated by glutamatergic signaling [6-8]. The glutamate system is used as the primary excitatory NT by pyramidal cells, the main excitable cells of the hippocampus [5,7]. For the larger part of an individual’s life, the chief inhibitory signaling in the hippocampus is mediated by GABAergic neurons, although it is notable that during early development GABA acts as an excitatory signal and only becomes inhibitory as development progresses [9]. Due to their expression patterns in pyramidal cells, the
subthreshold A- and M-currents can provide another source of inhibitory input by dampening the size of excitable responses in these cells [10,11].

The importance of the hippocampus in the CNS can be further observed in several neurological disorders wherein changes to hippocampal structure and function are often seen, some of which may be attributable to altered ion channel activity or expression. In particular, the identities of epilepsies and schizophrenia as potential 'channelopathies' will be explored in this thesis.

**Neurological diseases involving the hippocampal formation may be affected by the activity of subthreshold K+ currents.**

**Epilepsies**

A variety of epilepsies have been characterized, including familial neonatal seizures and temporal lobe epilepsy (TLE) [3,12]. Of the near 2% of individuals worldwide that are affected by epilepsy, the bulk of adult cases are TLEs [12]. TLE is, as the name suggests, localized to the temporal lobe of the brain, and largely involve the hippocampus [3,12].

For the most part, the onset of seizure activity in TLE appears to occur in the hippocampal formation and such TLEs are called mesial TLEs [3,12]. Such epilepsies often involve high frequency hippocampal oscillations called fast ripples that exist in the range of 200-400 Hz [10]. During fast ripples, it is not unusual for fast compound excitatory postsynaptic potentials (EPSPs) to be generated at postsynaptic cells, indicating that there is a lack of regulation of neuron excitability. Notably, the fast ripples are mainly seen in areas of seizure onset and so the fact that their presence has been observed in the hippocampal formation supports the key involvement of this brain region in TLEs [10]. Postmortem studies of TLE patient brains have revealed sclerosis of the hippocampus and, in fact, such significant losses of neuron volume that almost all of the neurons of the CA1 and CA3 are depleted [12]. This finding complicates the understanding of seizure genesis as such a severe reduction in neuron densities leaves few possible hippocampal sources for the onset of epileptic activity, although
the DG, which is involved in signaling pathways that traverse the CA1 and CA3, is one potential source that has been considered [12].

However, one must consider that recurring seizures in the hippocampus cause significant damage to it over time, thereby resulting in a progressive decline of neuronal function [12]. As neural wiring in this region is continually disrupted, the likelihood of decreased neuron functioning and signaling ability would go up, perhaps making the severity of the epilepsy [12]. It is possible, and has in some cases been established, that individuals with genetic mutations that alter the activity or expression of Kv channels may be predisposed to some forms of epilepsy [1-3]. Therefore, it is likely that mutations affecting a deficit in ion channel function, such as those that affect subthreshold K⁺ currents are also involved in epileptogenesis. The role of Iₘ, for example, has been widely studied as it relates to a form of epilepsy known as benign familial neonatal convulsions (BFNC) and further research has revealed that Iₘ may also have an important part to play in the development of other epilepsies [1-3,5].

**Schizophrenia**

Schizophrenia is a neuropsychiatric disorder that is characterized by positive symptoms, such as hallucinations and delusions, and negative symptoms, which includes impaired or disorganized cognition [13]. The positive symptoms, or psychosis, are typically the chief identifiers of schizophrenia or related disorders and are believed to involve alterations in D2-mediated dopamine signaling that mimic dopaminergic neurotransmission under the affects of psychostimulant drugs like amphetamines and cocaine [14,15]. Psychosis is typically treated with drugs that decrease the levels of striatal dopamine, however, there is no concrete evidence to suggest that there is a deficit in the dopamine system itself. Studies have therefore been investigating the possibility of alterations in other systems that may affect striatal dopamine [14,15].

One critical pathway points to the modulation dopaminergic signaling in the nucleus accumbens by excitatory hippocampal projections [14,15]. The pathway begins with
Dopaminergic neurons from the ventral tegmental area (VTA) that project into the nucleus accumbens of the striatum where they release this NT [14,15]. It is important to note that, at any given point under normal conditions, one half of the dopamine neurons here will be actively firing while the other half receive active inhibitory input from the GABAergic neurons of the ventral pallidum to the VTA [14,15]. The nucleus accumbens, the site of dopamine signaling, receives input from the ventral hippocampus in the form of excitatory glutamatergic neurons and the subsequent stimulation causes the nucleus accumbens to facilitate the inhibition of the ventral pallidum, thus allowing dopaminergic neurotransmission to occur [14,15]. An illustration of this pathway can be seen in figure 1-2 below.

**Figure 1-2: Dopamine signaling pathway involving the hippocampus.** Dopaminergic neurons of the VTA project into the nucleus accumbens of the striatum. Signaling of the dopamine neurons from the VTA is modulated by GABAergic inhibitory input from the ventral pallidum. When directly stimulated by excitatory hippocampal projections, the nucleus accumbens mediates the inhibition of GABA signaling in the ventral pallidum. As a result, dopamine signaling in the accumbens is increased.

The projections from the hippocampus to the accumbens consist of excitatory neurons known to express subthreshold $K^+$ currents and deficits in these currents are known to cause hyperexcitability. The implication of the pathway described above is that hyperexcitability of the
hippocampus, a feature that is seen in schizophrenia, could cause an increase in dopamine release in the striatum which would contribute to the development of psychosis [14,15].

It is also noteworthy that, like in epilepsies, the hippocampal formation in schizophrenic patients is reduced in terms of volume. However, unlike in epilepsy, the alterations to hippocampal volume do not appear to come about as the formation is progressively damaged. Instead, the rewiring and restructuring of the hippocampus can be observed not only as early as the onset of psychotic symptoms, but it can also be seen in individuals that are at high risk for developing this disease such as the identical twins of schizophrenic patients [9].

Also altered in the schizophrenic hippocampus is neural synchrony. Altered macroscopic gamma oscillations and their interplay with theta oscillations are both altered in this psychiatric disorder. Large-scale oscillatory activity in the hippocampus may be affected by alterations to neurons that disturb their intrinsic excitabilities, which can be done by enhancing or blocking ion channel currents [16]. Although a loss of GABAergic interneurons in the hippocampus may contribute to this, modifications to currents in pyramidal cells can also play a part [9]. Specifically, the role of $I_M$ in regulating hippocampal excitability makes it a candidate for involvement in the pathogenesis of schizophrenia.

Further exploration of the roles of these subthreshold $K_v$ channels in hippocampal neurons may offer insights into the molecular basis of neurological disorders such as epilepsy and schizophrenia. In this thesis, I will investigate the current literature regarding the functions of hippocampal $I_A$ and $I_M$ and how these subthreshold currents may affect the development of neurological disorders.
Chapter 2

Kv4 and the A-Type Current.

The A-type current is a subthreshold $K^+$ current that is chiefly produced by channels of the Kv4 family.

The A-type current is a transient outward $K^+$ current that is generated by channels with fast activation and inactivation kinetics as well as rapid recovery [5,6,10,17,18]. Such channels have widespread expression throughout the CNS and are functionally critical in the mammalian hippocampus. In hippocampal cells and networks, $I_A$ is involved in the regulation of neuronal excitability, synaptic plasticity, induction of LTP, and control of neurotransmitter release, specifically glutamate [5,6,10,17,18].

Synaptic plasticity, the temporal strengthening or weakening of a synapse, is thought to be a main component of memory formation. Induction of LTP at a synapse enhances the signaling occurring there, meaning that the synapse will be stronger than one not targeted for LTP [6-8]. A reduction in the amount of dendritic $I_A$ has been shown to lower the threshold for LTP. Conversely, the threshold for LTD, the process by which a synapse is weakened, is shown to be higher when $I_A$ is reduced [6-8].

The mammalian hippocampus harbors several different channels that produce the A-type current, but the most relevant to this thesis are those belonging to the Kv4 family. These channels produce a large fraction of the A-type currents that have a critical impact on dendritic excitability [19].

The expression patterns of Kv4 channels have a functional significance.

The somatodendritic localization of Kv4 expression is indicative of some of the primary roles of $I_A$ in hippocampal neurons. The chief subunits to consider in the composition of these channels are Kv4.2 and Kv4.3, though Kv4.2 alone is responsible for most of $I_A$ [20]. The expression of Kv4.2 is seen in pyramidal cells of the CA1 and CA3 regions, specifically in the dendritic spines of distal and proximal dendrites with very little expression in the soma. It should
be noted that Kv4.2 expression is markedly higher in the distal dendrites, as is the magnitude of $I_A$ produced here [20]. Kv4.2 is also seen in granule cell dendrites of the dentate gyrus where it coexpresses with Kv4.3 [20].

To address the importance of $I_A$ in the dendrites, it is important to first look at the physiological processes that occur normally in these compartments. The projections off of dendrites, known as dendritic spines, are the sites of synapse formation on the postsynaptic cell [19]. Here the neuron receives input from presynaptic neurons via neurotransmission, which then causes the generation of an action potential at the axon hillock of this postsynaptic neuron. When an action potential is generated, it is not only propagated down the axon but can also be backpropagated to the dendrites and dendritic spines where it excites the presynaptic neuron [20]. Dendrites are also the sites of postsynaptic potentials, which can be either excitatory (EPSPs) or inhibitory (IPSPs) depending upon the nature of the presynaptic neuron [20].

As a suppressor of excitatory stimulation, the presence of $I_A$ in the dendrites indicates that this current plays a role in regulating the backpropagation of action potentials, integration of synaptic signals, and synaptic plasticity [5,6,10,17,18,20]. The localization of Kv4.2 to dendritic spines puts these channels in an ideal position for: 1) modulating dendritic excitability and 2) being regulated by N-methyl-D-aspartate receptors (NMDARs) to allow for changes in the level of $I_A$ [5,6,10,17,18,20].

An important relationship exists between the activity of Kv4 channels and NMDAR composition.

The regulation of Kv4 channels by NMDARs has been thoroughly researched. NMDARs are one of two classes of glutamate receptors present at excitatory, glutamatergic synapses throughout the brain, the other class being α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA Rs) [6]. These receptors play an important part in the glutamate-mediated process of synaptic plasticity that occurs in the CA1 and CA3 regions of the hippocampus [5,6,10].
When an AP is generated in the axon initial segment it backpropagates to the soma and dendrites where synapses are located, including glutamatergic synapses [19]. The NMDA receptors that are located at such glutamatergic synapses are composed of NR1 and NR2 subunits [5-8]. The identity of the NR2 subunit, which tends to be either the NR2A or NR2B, determines a great deal of the receptor’s functionality [5-8]. During early postnatal mammalian development, synapses often feature NMDAR with a high ratio of NR2B to NR2A subunits, which suppresses the Kv4-generated $I_h$ and lowers the threshold for LTP induction by allowing for higher levels of glutamate release at a synapse [6-8].

The release of glutamate through NMDARs is important as the abundance of this NT not only upregulates LTP but also downregulates the total expression of Kv4.2 and causes a hyperpolarized shift in the channel’s inactivation [7,21]. It is notable that NMDARs are decidedly permeable to Ca$^{2+}$ and that the influx of Ca$^{2+}$ through these receptors is crucial for long-term modifications to synaptic plasticity [21]. Increases in Ca$^{2+}$ influx through these receptors is also an important mechanism of reducing Kv4.2 expression because it enhances the activity of Ca$^{2+}$-dependent proteases, like calpain, that may catalyze the break down of these K$^+$ channels [21]. The alteration of Kv4.2 activity can occur as NMDAR-mediated glutamatergic signaling can augment the activity of kinases such as extracellular signal-related kinase (ERK), protein kinase A (PKA), and protein kinase C (PKC), that can phosphorylate the Kv4 channels to reduce their activity [7].

It is also important to emphasize that Kv4.2 channels appear to have a regulatory effect on the subunit composition of NMDARs. Kv4.2 knockout (KO) mice have been shown to exhibit a high ratio of NR2B/NR2A subunits even after early development, thus displaying a delay of the synaptic maturation process in which the fraction of NR2A subunits usually increases [6]. The expression of EGFP-labeled Kv4.2 in these KO mice can restore dendritic $I_h$ and allow for synaptic maturation to progress, suggesting that Kv4.2 has some influence over NMDAR remodeling [6].
Relevance of the A-current to neurological diseases

Epileptogenesis and $I_A$

There is potential for Kv4 channels and $I_A$ to be involved in the development of epilepsies, particularly TLEs. Because the expression of Kv4 in pyramidal cells is distributed across the soma and dendrites of the CA1, CA3, and DG, these channels are particularly well placed to modulate dendritic excitability throughout the whole hippocampus [20]. Research using the Kv4.2 KO mouse model has shown that in cases of complete removal of Kv4.2, the A-type dendritic current is almost entirely abolished, thus synapses that normally express this channel are left vulnerable to hyperexcitable responses when stimulated [10,17]. It has also been demonstrated that dendritic $I_A$ is decreased in drug-induced models of TLE that use the convulsant drug pilocarpine [5].

A lack of dendritic $I_A$ can result not only in an increase in the size of backpropagated action potentials, but also the dysregulation of synaptic signal integration. Altering the activity or expression of Kv4 channels has been demonstrated to result in the fast, compound EPSPs that generate fast ripple oscillations [10]. These EPSPs can be characterized by their rapid rise, large size, and lasting for short periods of time. In the presence of normal Kv4 channel expression, the fast activating $I_A$ is able to suppress these types of EPSPs, thereby repressing seizure activity [10].

Also relevant to the role of Kv4 channels in the development of TLE is findings from postmortem studies that have shown that levels NMDAR mRNAs are upregulated in the epileptic hippocampus [22]. Specifically, levels of mRNAs encoding the NR2 subunits of NMDARs were significantly enhanced in TLE patients both with and without hippocampal sclerosis [22]. It is possible that this occurs as a result of disrupting the dynamic regulation that NMDARs and Kv4.2 exert over one another. The interplay between Kv4.2 and NMDARs has been established as being important in modulating LTP. If the function of Kv4.2 were compromised, perhaps through a genetic mutation, the threshold for LTP induction at
glutamatergic synapses would be lowered throughout the hippocampal formation. The result would be an excess of synapses with increased signaling strength that could persist over time and thus higher levels of glutamatergic neurotransmission would be present [5,6,10,17,18,20].
Chapter 3

Kv7 and the M-CURRENT

The M-current, a unique and critical subthreshold current, is produced by Kv7 channels.

The subthreshold M-type current is particularly notable for its unique properties and relevance to the pathophysiology of several neurological diseases. The generation of this current occurs around -60mV, which is close to physiological resting membrane potential. This unique activation range, coupled with the slow activation/deactivation kinetics and characteristic non-inactivation of channels that produce the M-current make this current unsuitable for contributing to the membrane repolarization of fast action potentials [2,9,11,23,24]. The functional importance of the M-current lies in its power to regulate the generation of action potential spikes, burst firing, neurotransmitter release, and neuronal synchrony throughout not only the hippocampus, but also proximal regions of the brain [2,9,11,23,24].

The expression of this current is widespread throughout regions of the CNS, including the hippocampal formation where the M-current is found chiefly in pyramidal glutamatergic neurons [9,11,23]. While the different classes of interneurons throughout the hippocampus may be known for burst firing, this property is not usually associated with pyramidal neurons [11]. This is likely a result of M-current expression as this particular K\(^+\) current is ideal for preventing burst firing of action potentials by making it more difficult to reach threshold [11]. In fact, the M-type current appears to be an important component of the collective K\(^+\) currents that are generated in pyramidal cells. The eradication of this current leads to hyperexcitability in these neurons, the consequences of which can be observed in the pathophysiologies of some diseases [9,11].

The channels that generate the M-type current have been identified as homotetramers and heterotetramers comprised of subunits from the Kv7 family. These channels exemplify the typical 6TM structure associated with voltage-gated K\(^+\) channels except that they have notably long C-terminal tails [9,23]. There are several members of this channel family that are
expressed specifically in the hippocampus include Kv7.2, Kv7.3, and to a smaller extent Kv7.5. Expression of the Kv7.2 and Kv7.3 subunits can be observed in the hippocampal *Mus musculus* slices seen in figure 3-1. In particular, the Kv7.2 and Kv7.3 subunits that are responsible for most of the M-current generation throughout the hippocampus are abundantly expressed in this region of the brain [9,11,23]. They have relatively similar expression patterns in the CA1-CA3 regions and the dentate gyrus. Particular attention will be paid in this chapter to the affects of the Kv7.2 and 7.3 subunits that are expressed in CA1 and CA3 pyramidal neurons.

Figure 3-1: Expression of Kv7 channels in the murine hippocampus. The expression of Kv7.2 (top) can be observed in the CA1, CA3, and to a smaller extent, the DG of this sagittal hippocampal slice scan. The expression pattern of Kv7.3 (bottom) is relatively similar as can be observed in the coronal hippocampal slice scan. Both brain scans were taken from the Allen Brain Atlas. Source: <http://www.brain-map.org/>.
The subcellular localization of Kv7 channels in pyramidal cells of CA1 and CA3 has been thoroughly explored and it has been found that the expression patterns of Kv7.2 and Kv7.3 have attributed to these channels the ability to modulate neuronal excitability both pre- and postsynaptically [25]. The channels have been shown to express, to an extent, at the soma and dendrites, but are notably most concentrated at the axon initial segment, a major site of action potential generation, and the nodes of Ranvier [25,26]. More specifically, clusters of Kv7.2/7.3 heteromers have been observed at the axon initial segment where these channels can regulate the shape of ADP waveforms and firing frequency [24]. Both Kv7.2 and Kv7.3 subunits have also been seen at the nodes of Ranvier all along the length of the axon where they likely contribute to action potential propagation and NT release at presynaptic terminals [24].

The activity of Kv7 channels can be adjusted via regulation by muscarinic agonists and PIP$_2$. Despite their activation by voltage-gating, Kv7.2/7.3 heteromers, which make up the bulk of the channels expressed, also need an adequate concentration of inner-membrane surface PIP$_2$ in order to open. Muscarinic agonists, in turn, suppress the M-current by causing the hydrolysis of available PIP$_2$ [9].

Acetylcholine receptors represent the most prominent cholinergic receptors in the CNS and are known for their importance in regulating other signaling systems in the brain, including dopaminergic signaling. While both M1 and M4 AChRs are expressed in the mammalian hippocampus, the primary regulation of Kv7.2/7.3 is mediated by the M1 receptor [9]. When a muscarinic agonist, such as oxotremorine-M, acts on the M1 receptor, the resultant activation couples with the G$_q$ signaling pathway. This pathway activates phospholipase-C$\beta$, the enzyme that mediates the breakdown of PIP$_2$, meaning that with the stimulation of M1-AChR activity there is a marked decrease in the membrane PIP$_2$ needed for Kv7.2/7.3 channel opening [9]. Therefore, modulation of the M-current mediated by PIP$_2$ and muscarinic agonists allows for the up- or downregulation of this K$^+$ current with respect to different situations.
Other methods of modulating Kv7 channel activity include the use of selective channel blockers and openers. Both linopiridine and XE991 are channel blockers that selectively bind Kv7 channels to reduce $I_M$ \[11,23,24,25\]. Despite the fact that both of these drugs have been shown to decrease Kv7 activity, they are not equally effective. XE991 is more potent than linopiridine \[24\]. Therefore, a lack of $I_M$ depletion with the application of linopiridine may in fact be a result of using too low a concentration. Experimenters may benefit from analyzing changes in $I_M$ and cell excitability under the separate influences of both drugs. The channel openers retigabine and, to a lesser extent NH6, have been shown to selectively augment the activity of Kv7 channels \[2,9,23,25\]. Retigabine in particular has been very useful in the study of Kv7 channels and their functional roles in the hippocampus, which will be explored later in this chapter.

Channels of the Kv7 family are distinctive in that they are they only producers of the M-type current, a current that is critically important in maintaining normal neuronal excitability. The following parts of this chapter will detail the importance of the M-current in modulating spike generation, burst firing, NT release, and neuronal synchrony, as well as how mutations that affect the properties of Kv7 channels can contribute to the pathophysiology of neurological disorders.

**Kv7 channels contribute to the modulation of spike generation and burst firing in pyramidal cells.**

The -55 to -60mV activation range of Kv7 is key to the regulation of action potential firing \[11,23\]. That these channels have an activation range so near to resting membrane potential, coupled with slow activation/deactivation kinetics and their characteristic non-inactivation, is indicative that the probability of channel opening is greater during prolonged depolarizations or in situations in which the neuronal firing frequency is high \[11,23,25\]. Unlike other K$^+$ currents, the M-current can contribute a large amount of K$^+$ efflux at the onset of action potential generation. While it was previously believed that the M-type current could play no role in
repolarization, more recent research has shown that Kv7 channels actually affect spike afterdepolarization (ADP), a critical determinant of burst firing [11,25].

Pyramidal neurons have the potential for burst firing which occurs when an excitatory stimulus causes not one but several successive spikes to be produced. In fact, these neurons have been shown to display both regular firing and burst firing in vivo with respect to different behavior states [11,25]. Aside from the M-current, factors that decide whether or not burst firing will occur include the persistent Na$^+$ current ($I_{NaP}$) and the size of spike ADP [11,25].

As membrane depolarization occurs with the influx of Na$^+$, the opening of K$^+$ channels progressively brings the membrane potential back to more negative voltages. As Kv7 channels are active at subthreshold ranges and have slow kinetics, they are unable to contribute to the initial repolarization and thus can have no significant affect on countering a quick depolarization [11]. However, during longer depolarizations as the membrane potential reaches physiologically relevant resting potential, enough Kv7 channels are able to open and allow for an increase in outward K$^+$ current that hyperpolarizes the cell [11,25]. Although a prolonged Na$^+$ influx would tend to favor a higher frequency of action potential firing the added current from Kv7 prevents the return to threshold and subsequent spikes, thereby resulting in a single spike [11].

It has been demonstrated that the chief cause of prolonged depolarizations of CA1 pyramidal neurons is the subthreshold activating $I_{NaP}$. This current is characterized by a long lasting flow of Na$^+$ into the cell that continues to generate depolarizing potentials after an action potential spike has occurred [11,25]. As the amount of $I_{NaP}$ increases so does the size of the resulting ADP, thereby increasing the probability of burst spikes being produced [11,23,25]. However, $I_{NaP}$ is not the only major contributor to spike ADP as $I_M$ activity can counter with a subthreshold activating efflux of K$^+$ [11,25].

By selectively blocking Kv7 channels the M-current can be reduced. During a long depolarization, this decrease in M-current results in a higher tendency of a neuron to generate burst spikes [11,25]. Experiments have shown that by using selective Kv7 channel blockers in
CA1 pyramidal cells, a significant part of the outward $K^+$ current that occurs after depolarization and at subthreshold potentials can be eradicated [11,23,24,25]. The importance of Kv7 is here highlighted because the $Na^+$ influx can no longer be sufficiently dampened by $K^+$ efflux, thus the $M$-current must be a vital contributor to the $K^+$ current. By regulating the activity of Kv7 in regions of the hippocampus, the alternation from single spike generation to burst spiking can be controlled [11,23,24,25]. This is critical when considering the fact that burst firing has much more potent downstream effects than regular firing, including the regulation of NT release [23].

The M-current affects neurotransmitter release not only in the hippocampus but in proximal brain regions as well, as can be seen in the dopamine system.

Because $I_M$ is so widely expressed throughout the hippocampus, which has connections to many other brain regions, this current is able to regulate a variety of NT systems at several levels. At synapses within the hippocampal formation, $I_M$ has been shown to inhibit the release of a variety of NT including GABA, norepinephrine, glutamate, D-aspartate, and acetylcholine [23,24,25,27]. However, the M-current can also regulate NT systems concentrated at other areas of the brain that are innervated by hippocampal projections [27]. The major focus of this section will be the regulation of dopamine signaling by hippocampal $I_M$.

First it is important to understand how the expression patterns of Kv7 channels allow for $I_M$-mediated control of NT release. The nodes of Ranvier, unsheathed areas of myelinated axons, have been established as sites of expression for Kv7.2 and Kv7.3 subunits [23]. Not only does this include regions of the axon near presynaptic terminals at which neurotransmitters are released [23,25]. As the generation of $I_M$ dampens the excitable response of the neuron, it may downregulate the level of neurotransmitter release. For example, the application of a Kv7 channel opener NH6 has been shown to reduce the frequency of mEPSCs at postsynaptic glutamatergic neurons and mIPSCs at postsynaptic GABAergic neurons, indicating that $I_M$ provided presynaptic inhibitory input [25]. As glutamatergic and GABAergic neurotransmission can be studied within the hippocampal formation itself, the effects of the M-current on these
processes are much easier to isolate. More difficult is elucidating the role of hippocampal $I_M$ in neurotransmission occurring outside of this brain region where there are complicated pathways involving various inputs and outputs to deal with. However, a critical pathway of dopamine signaling has been relatively well explored in which prominent hippocampal role in modulating the dopaminergic system [14,15].

This pathway, which was described in the introduction and can be observed in figure 1-2, suggests that the hippocampus is in a prime position to modulate levels of dopamine release and that hyperexcitability of the hippocampus may lead to excessive dopamine signaling in the nucleus accumbens [14,15]. The ability of Kv7 channels to influence dopaminergic signaling in the brain has been established in recent research. The channel opener retigabine was shown to reduce excitability in areas of the striatum, including the nucleus accumbens, and also decreased the response caused by the stimulant drugs cocaine and methylphenidate [23]. The decreased postsynaptic response to dopaminergic signaling that occurred with retigabine application was thought to be a result of a lessening of dopamine release. This is in keeping with the expected upregulation of $I_M$ due to the use of the Kv7 channel opener [23,27]. It has also been shown that the Kv7 channel blocker XE991 can eliminate the effect of retigabine on striatal dopamine levels [23]. This data is suggestive of the idea that $I_M$ produced by hippocampal pyramidal neurons can in fact have an effect on neurotransmission in proximal brain regions, provided there is direct input by the $I_M$-containing projections.

**Kv7 control of neuronal synchrony is seen in not only the current's effects on theta oscillations but also in its regulation of gamma oscillations.**

Neural oscillations can be described as rhythmic and repetitive patterns of electrical activity that are generated spontaneously and in response to specific stimuli throughout the central nervous system [28]. Although these oscillations can occur on the level of single neurons, the focus of this section will be the much larger-scale oscillations involving synchronized neuron networks. These phenomena have been observed in all mammals,
including humans, and appear to be important in neural coding as rhythms of different frequencies are related to different behavioral states and functions in the CNS [16,24,26,28,29]. The hippocampus mainly exhibits two different kinds of oscillations: theta and gamma [16,24,26,28,29].

While oscillations of a certain frequency may be evoked by neurons’ intrinsic excitability, these rhythms may also occur in response to the more complex stimuli. For example, some oscillations occur as a result of the dynamic interactions between neural networks or the combined affects of cells’ synaptic activities and intrinsic electrical properties, including the temporally-dependent currents produced by voltage-gated ion channels of the neurons. These more intricate mechanisms are believed to be the way in which hippocampal theta oscillations are generated [16,24,26,28,29].

Theta oscillations in the hippocampal region are slow, occurring in the 4-10 Hz frequency band, and have been observed via electroencephalogram (EEG) and magnetoencephalogram (MEG) recordings in both rodents and humans play a crucial part in regulating synaptic plasticity, cognitive processes such as learning and memory, and sensory-motor activity [16,24,26,28,29]. Previous research has established that of the three major currents in hippocampal pyramidal neurons of the CA1 that are responsible for establishing resonance in the theta range, two are $I_{NaP}$ and $I_M$ [24,26,29].

The importance of the interplay between the $I_{NaP}$ and $I_M$ currents in pyramidal cell has already been explored in this thesis with regards to burst firing but it should also be noted that these two currents are critical to a form of theta-resonance, called M-resonance, that functions at membrane voltages between those of resting membrane potential and AP spike threshold [26]. The role of $I_M$ in the generation of these theta rhythms was confirmed in *in vitro* experiments by blocking Kv7 channels with the bath application of 10mM XE991 to CA1 pyramidal neurons followed by current injection to hold the neurons’ membrane potentials at -63mV. The application of the Kv7 channel blocker at this subthreshold range eliminated theta
rhythms in the neurons and showed that the M-current contributes significantly to theta-oscillations in the hippocampus [26].

Less well studied are the effects of $I_M$ on gamma-oscillations in the hippocampus. These rhythms occur in the range of 30-100 Hz and it is understood that many aspects of hippocampal function, particularly synaptic plasticity, are augmented via the interplay of theta and gamma oscillations [24,29]. Signals that can cause the gamma oscillations to be produced in hippocampal circuits include the activation of muscarinic AChR, metabotropic glutamate receptors, and kainite receptors [24]. Gamma oscillations are initially produced in pyramidal neurons of the CA3 before they spread to the CA1 and so experiments in which the contribution of $I_M$ to these rhythms was analyzed were largely focused on activity in the CA3 [24].

The in vitro activation of kainite receptors while simultaneously blocking Kv7 channels was used in an experiment to study the effects of $I_M$ on gamma oscillatory activity. The application of the selective channel blockers XE991 and linopiridine were employed. This channel blocker not only lessened the power of the kainite-induced gamma rhythms, but also altered the firing pattern of CA3 pyramidal cells during these oscillations, thereby reducing the synchrony between field oscillatory activity and the characteristics of the pyramidal neuron firing [24]. Thus $I_M$ was shown to have a distinct effect on hippocampal gamma oscillations.

Relevance of the M-current to neurological diseases

The influence of $I_M$ in the development of neurological disorders has already been explored to a greater extent than that of many other voltage-gated ion channel currents. However, there is likely still more to be learned as research has continued to reveal possible roles for the M-current in the pathophysiology of neurological and neuropsychiatric diseases. In this thesis, the relationship of the M-current to epileptogenesis will be discussed as well as the potential involvement of this current in schizophrenia.

Epileptogenesis and $I_M$
The M-current generated by Kv7.2 and Kv7.3 channels has already been identified as playing a large part in the development of BFNC, a form of epilepsy in which clonic seizures occur by around three days of age and usually stop after three months [1,2]. Due to the importance of $I_M$ in modulating neuronal excitability, the sudden end of seizure activity seems at first strange, though it is likely related to the fact that GABAergic signaling is excitatory during early development and only becomes inhibitory as development progresses [25]. Due to these drastic changes that occur in GABA, the inhibitory effects of $I_M$ on excitability are likely much more important in the time period directly after an individual is born. Although most individuals with BFNC continue to develop normally after the first few months of life and experience no further seizure activity, at least 16% of people with this condition also suffer from either isolated or persistent seizures at some point after early development [2]. This is indicative of the fact that Kv7 channel mutations may, when compounded with other factors, still contribute to the development of seizures in adults. The crucial continued study of these channels has, fortunately, been made possible with the use of the specific channel opener retigabine.

The discovery of the link between BFNC and mutations in Kv7 was followed with the realization that the drug retigabine functions in opening these very channels that generate $I_M$ in the brain [1,2]. Retigabine causes a hyperpolarized shift in the activation ranges of Kv7 channels and alters the activation and deactivation kinetics by both speeding up channel activation and slowing deactivation [2,23]. It was found that this channel opener works by binding a tryptophan residue near the cytoplasmic part of S5, a residue that is conveniently missing in Kv7.1 which, unlike the other Kv7 subunits, is expressed only in cardiac tissues [2].

The anticonvulsant properties of retigabine have been studied in rodent models of epilepsy where this drug has shown success in suppressing seizures, particularly in young animals [2,23]. Retigabine has been widely used in research to study Kv7 channel function and has proved to be important in uncovering new, important influences that $I_M$ has on proper brain function, such as its potential link to schizophrenia.
Schizophrenia and $I_M$

Currently, there is little available research linking the Kv7 channels of the hippocampus to schizophrenia. However, the available literature regarding the function of hippocampal $I_M$, as well as its affects on dopamine signaling and neuronal synchrony reveal that this subthreshold current may well be involved in the pathophysiology of this disease.

One key piece of evidence involves the potential of $I_M$ to affect dopaminergic neurotransmission in the striatum. The neurons that project from the hippocampus into the nucleus accumbens are excitatory glutamatergic cells, some of which come from the ventral part of the CA1, and very likely express at least Kv7.2 and Kv7.3 [14,15,27]. That these neurons have a direct affect on the excitability of the accumbens suggests that modifications in their channel expression patterns and activities could compromise their regulation of dopamine signaling [14,15,23,27]. Furthermore, experiments that have shown that the application of retigabine results in decreased dopaminergic neurotransmission point to $I_M$ as having a significant influence over the excitable hippocampal input to the striatum [23,27].

One possibility is that the retigabine-induced suppression of $I_M$ was occurring in neurons from other brain regions, and not hippocampal projections. Expression of Kv7.2 and Kv7.3 in the striatum has been observed on neuron fibres, which were likely axonal projections into the striatum [23]. The dopamine projections to the striatum are unmyelinated and, as Kv7 near presynaptic terminals is usually seen in the nodes of Ranvier of myelinated cells, were probably not the sites of Kv7 expression [23]. It is therefore very possible that the projections that expressed Kv7.2 and Kv7.3 were hippocampal pyramidal cells.

Another factor that links hippocampal $I_M$ to schizophrenia is this current’s potential to alter oscillatory activity. Schizophrenic patients have exhibited a decrease in the synchronization of gamma oscillatory activity [16,24,29]. What’s more, it has been established that proper cross-frequency coupling of gamma and theta oscillations is also disturbed [16,24,29]. Combined with structural changes to the hippocampal formation, inconsistencies in these rhythms can influence...
the altered cognition that is observed in schizophrenia [13]. The CA1 and CA3 pyramidal cells are critical to the generation of these oscillations and a loss of inhibitory input to these neurons from GABAergic interneurons may compromise the hippocampal rhythms [9,13,16]. However, it is also possible that alterations to the intrinsic excitabilities of the pyramidal cells themselves via reduction of $I_M$ may compound the issue, as is evidenced by experiments in which the application of Kv7 channel blockers to hippocampal slices disrupted neural synchrony, whereas the restoration of $I_M$ to pyramidal neurons restored oscillatory activity to normal [24].

At the very least, the available research shows that Kv7 channel openers and blockers may be useful for observing the effects of $I_M$ on amphetamine-stimulated dopamine release in mice. Establishing that a lack of $I_M$ can be a component of schizophrenia would give insight into the molecular pathophysiology of this debilitating disease and potentially allow for the development of novel treatments for psychosis.
Conclusion

The functional characteristics of the hippocampal $I_A$ and $I_M$ currents have been thoroughly explored in previous research. However, there is still much work that needs to be done in terms of investigating how these currents can affect neurological disorders. In particular, this thesis explored the involvement of these currents in the pathophysiologies of epilepsies and schizophrenia.

Due to their importance in regulating pyramidal cell excitabilities in the hippocampal formation, both $I_A$ and $I_M$ should continue to be studied with regard to their abilities to contribute to epileptogenesis. There is also compelling evidence to suggest that these currents may be involved in other diseases of the CNS, particularly in the case of $I_M$. Further elucidating the role of Kv7-generated $I_M$ in schizophrenia, for example, could provide new insights into the pathophysiology of this psychiatric disorder.

Currently, limitations in mouse models of psychiatric diseases make the modeling of psychosis difficult in a laboratory setting. However, altering the strength of amphetamine-stimulated dopamine release in mice with the drugs retigabine, XE991, and linopiridine might provide an interesting way in which the relationship between $I_M$ and schizophrenia could be studied. Furthermore, if augmenting hippocampal $I_M$ is able to effectively reduce striatal dopamine levels, then this may pave the way for potential new treatments of psychotic symptoms.
Bibliography.


