Functions of voltage- and calcium-activated potassium channels in the hippocampus and medial entorhinal cortex

Doctoral thesis by

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LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by the Roman numerals in the text.

**PAPER I**


**PAPER II**

HÖNIGSPERGER C, NIGRO MJ, STORM JF. Physiological roles of Kv2 channels in entorhinal cortex layer II stellate cells revealed by Guangxtoxin-1E. *Submitted to Journal of Physiology.*

**PAPER III**

MATEOS-APARICIO P, HÖNIGSPERGER C, STORM JF. Dorsoventral differences in the sAHP and excitability of dentate gyrus granule cells of rats and mice. *Manuscript.*

**PAPER IV**

WANG K, MATEOS-APARICIO P, HÖNIGSPERGER C, RAGHURAM V, WU W, RIDDER MC, SAH P, MAYLIE J, STORM JF, ADELMAN JP. IK1 channels do not contribute to the slow afterhyperpolarization in pyramidal neurons. Accepted for publication in *eLife.*
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACSF</td>
<td>Artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>ADP</td>
<td>After-depolarization</td>
</tr>
<tr>
<td>AHP</td>
<td>After-hyperpolarization</td>
</tr>
<tr>
<td>BLA</td>
<td>Basolateral amygdala</td>
</tr>
<tr>
<td>BK</td>
<td>Large conductance Ca$^{2+}$-activated K$^+$ channel</td>
</tr>
<tr>
<td>CA1</td>
<td>Cornus ammonis 1</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>ChTx</td>
<td>Charybotoxin</td>
</tr>
<tr>
<td>DGC</td>
<td>Dentate granule cell</td>
</tr>
<tr>
<td>D-V</td>
<td>Dorsoventral</td>
</tr>
<tr>
<td>fAHP</td>
<td>Fast after-hyperpolarization</td>
</tr>
<tr>
<td>f/I</td>
<td>Firing rate versus current input</td>
</tr>
<tr>
<td>GTx</td>
<td>Guangxitoxin-IE</td>
</tr>
<tr>
<td>$I_M$</td>
<td>Kv7/M potassium current</td>
</tr>
<tr>
<td>$I_K$</td>
<td>Delayed rectifier potassium current</td>
</tr>
<tr>
<td>IK1</td>
<td>Intermediate conductance calcium-activated potassium current</td>
</tr>
<tr>
<td>IR-DIC</td>
<td>Infrared-differential interference contrast</td>
</tr>
<tr>
<td>$I_{AHP}$</td>
<td>Slow after-hyperpolarization current</td>
</tr>
<tr>
<td>Kv</td>
<td>Voltage-gated potassium channel</td>
</tr>
<tr>
<td>L II</td>
<td>Layer II</td>
</tr>
<tr>
<td>mAHP</td>
<td>Medium after-hyperpolarization</td>
</tr>
<tr>
<td>mEC</td>
<td>Medial entorhinal cortex</td>
</tr>
<tr>
<td>sAHP</td>
<td>Slow after-hyperpolarization</td>
</tr>
<tr>
<td>SC</td>
<td>Stellate cell</td>
</tr>
<tr>
<td>SK</td>
<td>Small conductance calcium-activated potassium channel</td>
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INTRODUCTION

Overview
The neuron (nerve cell) is a specialized cell type within the nervous system, which receives information in the form of electrical and chemical signals from other neurons. These signals can change the neuronal membrane potential and initiate an action potential (spike) after passing a certain voltage threshold. The action potential is then rapidly propagated along the axon and passes signals to other neurons. More than 60 years ago Hodgkin and Huxley discovered the ionic mechanisms underlying the generation and propagation of action potentials in the giant squid axon (Hodgkin & Huxley, 1952). The rapid depolarization and repolarization during an action potential is mediated by flow of Na$^+$- and K$^+$- currents through the neuronal membrane. Later it has been shown that neuronal membranes contain ion channels that are selectively permeable for different ionic species and have highly diverse structures and functions (Hille, 2001). Hippocampal pyramidal cells, for example, exhibit multiple K$^+$ currents which contribute differently to the 1) resting membrane potential, 2) subthreshold behavior, 3) spike repolarization, and 4) after-potentials following spikes (Storm, 1990). As a consequence, these pyramidal cells can fire over a wide range of frequencies in response to varying current inputs, and exhibit complex voltage responses and firing patterns.

In the present thesis, I investigated the functions of different voltage- and calcium-activated K$^+$ currents in the hippocampus and medial entorhinal cortex including Kv7/M current ($I_{M}$) (paper I), Kv2 current ($I_{K}$) (paper II) and the current mediating the slow after-hyperpolarization following a spike train (sAHP, $I_{sAHP}$) (paper III, IV).

These currents have important roles in modulating neuronal excitability and other intrinsic electrophysiological properties (Storm, 1990), and are involved in epilepsy and other neurodegenerative diseases (Jentsch, 2000; Shah & Aizenman, 2014).

The following section summarizes several functional roles of K$^+$ currents in neurons.

Spike repolarization
The rapid depolarization during a spike is caused by activation of Na$^+$ channels, followed by a repolarizing phase due to fast inactivation of Na$^+$ channels and activation of K$^+$ channels, as described by the original work of Hodgkin & Huxley in the giant squid axon (Hodgkin & Huxley, 1952). Later, it was shown that a variety of K$^+$ channels contribute to the somatic spike repolarization in central mammalian neurons including large conductance Ca$^{2+}$-activated K$^+$ channels (BK channels), Kv4 channels and Kv3 channels (Storm, 1990; Bean, 2007).

Interestingly, in many mammalian central neurons, Kv1 channels have little or no effect on somatic spike repolarization but contribute to spike repolarization in axons of layer 5 pyramidal cells (Kole et al., 2007) and axonal terminals of dentate granule cells (Geiger & Jonas, 2000; Alle et al., 2011).
Kv2 channels can also contribute to somatic spike repolarization. Thus, knock-down of Kv2 mRNA in cultured CA1 pyramidal cells increased spike widths during high frequency synaptic stimulation (Du et al., 2000). Furthermore, block of Kv2 channels by Guangxitoxin-1E in acutely isolated CA1 pyramidal cells and sympathetic neurons can increase the width of the single spikes and reduce maintained firing (Liu & Bean, 2014). In contrast, expression of a dominant negative Kv2 pore mutant in cultured cortical pyramidal cells had no effects on spike repolarization (Guan et al., 2013).

In Paper II, we studied the role of Kv2 channels in medial entorhinal cortex (mEC) layer II stellate cells using the novel Kv2 blocker Guangxitoxin-1E (Herrington et al., 2006; Liu & Bean, 2014). We found that Kv2 channels regulate spike repolarization during repetitive firing but had little or no impact on the first spike of the train.

**Spike afterpotentials**

A characteristic feature of many neurons is to adapt and decrease spike firing in response to prolonged stimulation. This adaptation is partly caused by outward currents underlying after-hyperpolarizations (AHPs) that follow repetitive spike firing and counteract the excitatory drive of a depolarizing stimulation (Calvin & Schwindt, 1972; Madison & Nicoll, 1984; Storm, 1990).

In CA1 pyramidal cells, a spike or a spike train is usually followed by a sequence of AHPs: a fast AHP (fAHP) lasting 2-5 ms, a medium AHP (mAHP) lasting 50-100 ms, and a slow AHP (sAHP) lasting more than 1 s (Storm, 1987; Storm, 1989; Storm, 1990). Increasing the stimulation intensity and the number of spikes per train causes an increase in the corresponding mAHP and sAHP which limits spike firing and serves as a “brake” during strong repetitive discharge. Between fAHP and mAHP, spikes can also be followed by an after-depolarization (ADP) lasting 20-40 ms (Storm, 1987; Jensen et al., 1996). If the ADP crosses the spike threshold, it can initiate another spike followed by another ADP, and so on, thus generating a burst (spike cluster). Bursts are a characteristic mode of spike firing in vivo (Kandel & Spencer, 1961), and are thought to be more important for reliable neural coding than single spikes (Lisman, 1997).

Until now a variety of membrane currents that contribute to AHPs and the ADP in central neurons have been discovered.

**Fast after-hyperpolarization (fAHP)**

Ca\(^{2+}\)-activated K\(^+\) current of the BK type (\(I_C\)) contributes to the somatic fAHP and spike repolarization in a variety of neurons (Adams et al., 1982; Storm, 1987; Shao et al., 1999; Hu et al., 2001; Faber & Sah, 2003). In contrast, dendritic recordings in CA1 pyramidal cells suggested that \(I_C\) does not affect the waveform of back-propagating spikes, due to a lack of \(I_C\) in dendritic compartments (Poolos & Johnston, 1999). Poolos & Johnston (1999) suggested that the lack of dendritic \(I_C\) in the denrites may contribute to slow spike repolarization and increased Ca\(^{2+}\) influx during back-propagating spikes and synaptic stimulation.
Recordings from axon terminals in hippocampal mossy fibers revealed fast spike repolarization and a prominent fAHP (Geiger & Jonas, 2000), which are mediated by Kv1 and Kv3 channels under basal conditions (Alle et al., 2011). However, when these and other Kv channels are blocked, BK channels can repolarize pre-synaptic spikes and thereby limit transmitter release (Hu et al., 2001; Alle et al., 2011).

In Paper II we demonstrated in mEC layer II stellate cells that also Kv2 channels contribute to the somatic fAHP during repetitive firing, but not following a single spike.

Medium after-hyperpolarization (mAHP)
In CA1 pyramidal cells Kv7/M-channels and HCN/h channels underlie the mAHP at subthreshold and hyperpolarized potentials, and control excitability and spike frequency adaptation (Storm, 1989; Gu et al., 2005).

In other cell types calcium-activated $K^+$ channels of the SK type mainly contribute to the mAHP and thereby play a similar role in controlling repetitive firing patterns (Faber & Sah, 2007; Faber, 2009; Vogalis et al., 2003; Mateos-Aparicio et al., 2014; Gu et al., 2008).

In Paper I we investigated the properties of Kv7/M-channels in CA1 pyramidal cells along the dorso-ventral hippocampal axis. We found that dorsal pyramidal cells show a bigger, more readily activated Kv7 current, a larger Kv7-dependent mAHP, and a stronger impact of other Kv7-related properties than ventral pyramidal cells.

In Paper II we demonstrated that Kv2 channels can contribute to the somatic mAHP during repetitive firing in mEC layer II stellate cells.

Slow after-hyperpolarization (sAHP)
The sAHP in hippocampal CA1 pyramidal neurons is attributed to a slow Ca$^{2+}$-activated $K^+$ current (Hotson & Prince, 1980; Gustafsson & Wigström, 1981; Lancaster & Adams, 1986) that controls late spike rate adaptation and excitability during repetitive firing (Madison & Nicoll, 1984; Storm, 1990; Faber & Sah, 2002; Vogalis et al., 2003). The sAHP is strongly reduced by a variety of neuromodulators such as monoamines and peptides that act through common intracellular signaling cascades including cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), protein kinase C (PKC) and phospholipase C (Madison & Nicoll, 1982; Madison & Nicoll, 1986; Pedarzani & Storm, 1993; Pedarzani & Storm, 1995; Vogalis et al., 2003).

Despite long-standing research, the molecular mechanisms underlying the sAHP remain controversial (Andrade et al., 2012). Recent findings suggest that multiple types of potassium channels may contribute to the sAHP, which are indirectly gated via diffusible Ca$^{2+}$-sensors and an increase in phosphatidylinositol 4,5-bisphosphate (Andrade et al., 2012).

Recently it was reported that the sAHP and the IsAHP were partially reduced in CA1 pyramidal neurons by TRAM-34, which blocks intermediate-conductance Ca$^{2+}$-activated $K^+$ channels (SK4, IKCa, KCa3.1), and the sAHP was reported to be absent in CA1 pyramidal
neurons from IK1 null mice, strongly suggesting that IK1 channels underlie the sAHP (King et al., 2015).

In Paper IV we have investigated the sensitivity of the sAHP and the IsAHP to TRAM-34 in pyramidal neurons of CA1 and the basolateral amygdala. We found no evidence that TRAM-34 affects the sAHP and IsAHP measured in current and voltage clamp. In addition, IK1 knock-out mice also exhibited a characteristic IsAHP.

In Paper III we investigated the electrophysiological properties of dentate granule cells along the hippocampal dorsoventral axis and found that dorsal granule cells show a bigger sAHP and reduced excitability compared to ventral granule cells.

After-depolarization (ADP)
In CA1 pyramidal cells block of the persistent sodium current (\(I_{NaP}\)) (Azouz et al., 1996) and the R-type calcium current (Metz et al., 2005) reduce the ADP. Conversely, block of different voltage-activated \(K^+\) currents including A-type \(K^+\) current (\(I_A\)) (Magee & Carruth, 1999), D-type \(K^+\) current (\(I_D\)) (Metz et al., 2005), and Kv7/M-type \(K^+\) current (\(I_M\)) (Yue & Yaari, 2004), increases the ADP and facilitates bursting.

Little is known how Kv2 channels regulate firing patterns in central neurons.

In Paper II we found that block of Kv2 channels by Guangxitoxin-1E increased the ADP and facilitated bursting in mEC layer II stellate cells.

Resonance
Electrical oscillations in brain networks are generated by coherent activation of a large number of neurons and are associated with specific behavioral states (Steriade et al., 1993). Theta oscillations are a prominent type of network oscillation found e.g. in the hippocampus in the form of rhythmic activity in the theta frequency band. They are present during locomotion and REM sleep (Buzsáki, 2002).

Network oscillations depend in principle on the synaptic connections of the neuronal network and on intrinsic properties of the single neurons. It has been shown that neurons in different brain areas generate intrinsic oscillatory voltage responses, and respond best to oscillatory inputs at certain frequencies called resonance (Hutcheon & Yarom, 2000). The resonance properties are generated by an inter-play of passive and active membrane properties. The passive properties include the capacitance and leak conductance that act as a low-pass filter, thereby attenuating the effects of high-frequency inputs. In contrast, active properties are determined by specific voltage-gated currents which attenuate inputs at low frequencies. The combination of both mechanisms generates a band-pass filter that enables the strongest voltage response at a certain frequency (resonance frequency).

Voltage-gated currents that mediate resonance include \(Ca^{2+}\) current (\(I_T\)) in olivary neurons (Lampl & Yarom, 1997) and thalamic neurons (Puil et al., 1994), HCN/h current (\(I_h\)), TEA-sensitive \(K^+\) current and persistent sodium current (\(I_{NaP}\)) in neocortical pyramidal neurons (Gutfreund et al., 1995; Hutcheon et al., 1996).
CA1 pyramidal show $I_{H}$- and $I_{M}$-dependent resonance (H-resonance and M-resonance) at hyperpolarized and depolarized membrane potentials, respectively, and in particular M-resonance can be amplified by $I_{NaP}$ (Hu et al., 2002; Hu et al., 2009).

Recent studies suggested that $I_{H}$-dependent resonance varies in CA1 pyramidal cells along the dorsoventral axis (Marcelin et al., 2012a; Dougherty et al., 2013).

In Paper I we also investigated whether $I_{M}$ contributes to differences in resonance properties, and found stronger $I_{M}$-dependent resonance in dorsal than in ventral CA1 pyramidal cells.

**Kv7/M channels**

The M current ($I_{M}$), mediated by M-type voltage-gated $K^{+}$ channels, was first described in frog sympathetic ganglion neurons, and is inhibited by muscarinic acetylcholine receptor (mAChR) agonists (hence the name “M current”; Brown & Adams, 1980). Later, $I_{M}$ was also found in mammalian hippocampal pyramidal cells and in other neurons (Halliwell & Adams, 1982; Schroeder et al., 2000a; Brown & Passmore, 2009). $I_{M}$ activates at subthreshold potentials, from about -60mV, it has slow activation and deactivation kinetics (tens of milliseconds), and does not inactivate. As a consequence, the sustained activity of $I_{M}$ below spike threshold plays an important role in reducing cellular excitability in many neuronal cell types (Storm, 1990; Marrion, 1997).

Later studies found that $I_{M}$ is carried by heteromeric combinations of Kv7.2 and Kv7.3 channels (encoded by KCNQ2/3 genes) in sympathetic ganglion neurons (Wang et al., 1998), with a possible contribution of Kv7.5 (KCNQ5) in other neurons (Schroeder et al., 2000a; Shah et al., 2002; Tzingounis et al., 2010).

A subsequent important discovery was that muscarinic inhibition of $I_{M}$ depends on activation of phospholipase C (PLC) and a decrease in membrane phosphatidylinositol 4,5-bisphosphate (PIP$_{2}$) levels (Suh & Hille, 2002; Zhang et al., 2003).

In CA1 pyramidal cells, inhibition of $I_{M}$ can regulate multiple cellular properties including spike frequency adaptation (Madison & Nicoll, 1984), the medium after-hyperpolarization (Storm, 1989; Storm, 1990; Gu et al., 2005; Gu et al., 2008), the after-depolarizations and burst-firing (Yue & Yaari, 2004), the spike threshold (Shah et al., 2008), and the theta resonance at depolarized potentials (M-resonance) (Hu et al., 2002; Hu et al., 2009). In hippocampal and neocortical axons, $I_{M}$ can regulate pre-synaptic excitability and transmitter release (Vervaeke et al., 2006; Sun & Kapur, 2012), M-resonance (Alle et al., 2009), and the resting membrane potential (Battefeld et al., 2014).

Kv7 channels are encoded by the KCNQ gene family, which contains 5 members (KCNQ1-5; Kv7.1-7.5). Mutations in 4 out of 5 genes are associated with diseases including cardiac diseases and arrhythmia/congenital deafness (KCNQ1; long-QT syndrome), neonatal epilepsy (KCNQ2/3; benign familial neonatal convulsions), and progressive hearing loss (KCNQ4) (Jentsch, 2000).

The KCNQ subunits are structurally related to other Kv subunits and have 6 transmembrane segments that contain a pore-forming P-loop and a positively charged voltage sensor (Jentsch,
Together, four KCNQ subunits form a functional channel that can assemble as hetero- or homomers depending on the individual subunits (Jentsch, 2000).

The KCNQ/Kv7 subunits have different expression patterns and functions: KCNQ1, associated with β-subunit KCNE1, mediates a delayed rectifier K⁺ current which repolarizes cardiac action potentials (Sanguinetti et al., 1996) and mediates trans-epithelial K⁺-flux in the inner ear (Vetter et al., 1996). In the intestine, KCNQ1 can also assemble with KCNE3, mediating a fast-activating, constitutively open K⁺ current involved in chloride secretion (Schroeder et al., 2000).

KCNQ2/3 are the main subunits underlying IM and show overlapping expression patterns in various brain areas including sympathetic ganglia (Wang et al., 1998), neocortex and hippocampus (Cooper et al., 2000; Battefeld et al., 2014). At the subcellular level, Kv7.2 and Kv7.3 bind to Ankyrin G, which mediates co-localization of Kv7 channels with voltage-gated Na⁺ channels at the axon initial segment (Pan et al., 2006). Kv7.2 and Kv7.3 channels are also highly expressed in axonal nodes of Ranvier (Devaux et al., 2004; Pan et al., 2006). Conditional suppression of KCNQ2 in mice cause cortical hyperexcitability and seizures (Peters et al., 2005; Soh et al., 2014), but no or only modest effects are observed in mice with suppression of KCNQ3 (Soh et al., 2014). It has been reported that β-secretase BACE1, involved in Alzheimer’s disease, can associate with Kv7/M channels as a β-subunit, thereby amplifying IM and changing its kinetics (Hessler et al., 2015).

KCNQ4 is expressed in sensory outer hair cells, and in brain stem nuclei in the central auditory pathway. Thus, they are involved in a dominant form of progressive hearing loss (Kubisch et al., 1999; Kharkovets et al., 2000). Alternative splice variants of KCNQ4 channels have been reported, and they differ in their voltage-dependence and modulation by the Ca²⁺-binding protein calmodulin (Xu et al., 2007).

KCNQ5 is expressed throughout the mammalian brain and can contribute to IM (Lerche et al., 2000; Schroeder et al., 2000a; Shah et al., 2002; Tzingounis et al., 2010). Association with β-subunit KCNE1 can slow activation and increase Kv7.5 currents, while KCNE3 inhibits Kv7.5 currents (Roura-Ferrer et al., 2009). At the subcellular level, KCNQ5 is localized to postsynaptic sites of inhibitory synapses, and controls excitability and network functions through synaptic inhibition (Fidzinski et al., 2015).

Investigations of the Kv7 channels are presented in Paper I.

**Kv2 channels**

The cDNA of rat Kv2.1 (rat: drk1, human: KCNB1) was initially isolated by expression cloning in oocytes. These channels mediate a delayed rectifier K⁺ current (I_K) with a high activation threshold (-15 mV) and slow activation kinetics (Frech et al., 1989). [The term “delayed rectifier” usually refers to a broader class of K⁺ currents which are similar to the kinetics and voltage-dependence of the K⁺ -current in squid axon described by Hodgkin & Huxley (1952), and it is distinct from the typical “A-type” K⁺ current that has faster activation and inactivation kinetics (Rudy, 1988; Storm, 1990).]
The second member of the Kv2 family, Kv2.2 (rat: cdrk, human: KCNB2) shows similar kinetics as Kv2.1. It can form functional heteromers with Kv2.1 in oocytes (Blaine & Ribera, 1998).

Both Kv2.1 and Kv2.2 K⁺ channels are widely expressed in the nervous system, but show different cellular or subcellular distributions (Trimmer, 1991; Hwang et al., 1993; Maletic-Savatic et al., 1995). Noteworthy, Kv2.1 channels form clusters on the soma and the proximal dendrites of pyramidal cells and interneurons adjacent to astrocytic processes (Scannevin et al., 1996; Du et al., 1998). Kv2.1 channel clusters are also found in the axon initial segment of cultured hippocampal pyramidal cells (Sarmiere et al., 2008).

Kv2.1 channels mediate a delayed rectifier current, $I_{K_d}$, in cultured rat hippocampal pyramidal neurons (Murakoshi & Trimmer, 1999) and globus pallidus neurons (Baranauskas et al., 1999). Knock-down of Kv2.1 mRNA in cultured CA1 pyramidal neurons revealed a frequency-dependent increase in spike broadening and Ca²⁺ entry during synaptic stimulation (Du et al., 2000). Transient expression of Kv2 pore mutants also reduced spike repolarization in cultured sympathetic neurons (Malin & Nerbonne, 2002), and reduced maintained repetitive firing in these neurons and in pyramidal neurons from the motor cortex (Malin & Nerbonne, 2002; Guan et al., 2013). A selective Kv2 blocker, Guangxitoxin-1E (GTx), has been isolated from tarantula venom. GTx broadens spikes in pancreatic β-cells, thereby enhancing glucose-dependent insulin secretion (Herrington et al., 2006). In isolated CA1 pyramidal neurons and sympathetic neurons, application of GTx reduced spike repolarization, depolarized inter-spike trajectories, and disrupted repetitive firing (Liu & Bean, 2014).

Several lines of evidence suggest that Kv2 channels are involved in homeostatic regulation of neuronal excitability in normal and diseased brains (Misonou et al., 2005; Misonou, 2010; Shah & Aizenman, 2014). Under basal conditions the cytoplasmatic C-termini of Kv2.1 channels are highly phosphorylated, but can be de-phosphorylated by Ca²⁺ influx and activation of the Ca²⁺/calmodulin-dependent phosphatase calcineurin (Misonou et al., 2004; Misonou et al., 2005; Park et al., 2006). As a consequence, the de-phosphorylation disrupts the clustered localization of Kv2.1 and shifts its activation threshold to more negative potentials (Misonou et al., 2004). This reduces neuronal excitability in cultured neurons in an activity-dependent manner (Mohapatra et al., 2009). Kv2 channels may therefore have a neuroprotective role by dampening neuronal excitability (Misonou et al., 2005). In contrast, Kv2.1 can also trigger apoptotic cell death through K⁺ efflux induced by oxidants and staurosporine in cortical neurons (Pal et al., 2003).

It has been shown that the activity of Kv2.1 are modulated by a variety of signals including: 1) activation of ionotrophic glutamate receptors during kainate-induced seizures (Misonou et al., 2004; Misonou et al., 2005); 2) activation of muscarinic receptors (Mohapatra & Trimmer, 2006); 3) during ischemia and activation of cyclin e1 (Misonou et al., 2008; Shah et al., 2014); 4) small ubiquitin like-proteins (Plant et al., 2011); and 5) nitric-oxide associated pathways (Steinert et al., 2011).

Kv2.1 channels may also have non-electrical properties. In cultured hippocampal pyramidal neurons, about 70 % of Kv2.1 channels are non-conducting, and their conductance depends on surface channel density (Fox et al., 2013). Especially Kv2.1 clusters may serve as platforms for delivery of other Kv-channels and proteins (Deutsch et al., 2012).
It has also been reported that Kv2.1 channels promote exocytosis by interactions with the SNARE protein syntaxin in neuroendocrine cells (Singer-Lahat et al., 2007) and dorsal-root-ganglion neurons (Feinsreiber et al., 2010).

Investigations of the Kv2 channels are presented in Paper II.

**Ca²⁺-activated K⁺ channels**

Ca²⁺-activated K⁺ channels are opened by increases in intracellular Ca²⁺ levels, in contrast to voltage-dependent K⁺ channels such as Kv7/M channels and Kv2 channels, as described above. However Ca²⁺-activated K⁺ channels of the BK-type are also voltage-dependent and open during membrane depolarizations, even when intracellular Ca²⁺ levels are constant (Hille, 2001). Ca²⁺-activated K⁺ channels were initially discovered in molluscan neurons (Meech, 1974) and were later also found in many vertebrate cell types (Adams et al., 1982; Rudy, 1988; Latorre et al., 1989; Storm, 1990).

Later, different subtypes of Ca²⁺-activated K⁺ channels were described, with different single channel conductance: small-conductance (SK, 4-14 pS), intermediate-conductance (IK, 20-80 pS) and big-conductance (BK, 100-250 pS) (Vergara et al., 1998; Hille, 2001). SK, IK, and BK channels can also be distinguished by their voltage-dependence, Ca²⁺ sensitivity and pharmacology (Hille, 2001).

SK channels are blocked by a number of pharmacological agents including the bee venom apamin (Hugues et al., 1982; Blatz & Magleby, 1986), some bicuculline salts (Johnson & Seutin, 1997) and the scorpion toxin scyllatoxin (Castle & Strong, 1986).

SK channels constitute of three subtypes SK1-3 (KCa2.1-3) encoded by KCNN1-3 (Köhler et al., 1996), and have 6 transmembrane domains with a pore forming P loop region. The different SK channel subtypes are widely expressed in the brain, where SK1-2 are prominently expressed in the neocortex and hippocampus, and SK3 are predominantly found in phylogenetically older brain areas including basal ganglia, thalamus and the brain stem (Stocker & Pedarzani, 2000; Sailer et al., 2002; Sailer et al., 2004). At the subcellular level, SK1-3 are localized to somato-dendritic compartments and varicose fibers (Sailer et al., 2002; Sailer et al., 2004; Ballesteros-Merino et al., 2014).

SK channels can contribute to the mAHP and reduce repetitive firing patterns in different neuronal cell types (Vogalis et al., 2003; Faber & Sah, 2007; Faber, 2009; Mateos-Aparicio et al., 2014). [Previously it was suggested that SK channels mainly contribute to the sAHP. However this possibility was discarded by later studies (Faber & Sah, 2007; Andrade et al., 2012).]

In addition SK channels were found to regulate dendritic integration and synaptic plasticity in CA3-CA1 Schaffer collateral synapses (Gu et al., 2008; Lin et al., 2008), and enhance memory formation after block of SK channels with apamin (Stackman et al., 2002).

The intracellular C-terminus of SK channels contains protein kinase A dependent phosphorylation sites, which mediate reduction of SK2 surface expression in COS7 cells (Ren et al., 2006), in pyramidal neurons in the lateral amygdala through activation of β-adrenergic
receptors (Faber et al., 2008), and in hippocampal pyramidal neurons after induction of long-term-potentiation (LTP) (Lin et al., 2008).

SK channels have been suggested as targets for treatment of neurological disorders like schizophrenia, depression, and Parkinson’s disease (Faber, 2009).

IK channels (SK4, IKCa or KCa3.1) were first cloned from the human pancreas, and are blocked by charybdoctoxin and TRAM-34 (Ishii et al., 1997; Wulff et al., 2000), but are weakly affected by SK channel blocker apamin and BK channel blocker iberiotoxin (Ishii et al., 1997). IK channels mediate currents similar to the originally described “Gardos channels” in erythrocytes (Gardos, 1958). They are additionally found in myenteric neurons in the intestine (Nguyen et al., 2007) lymphocytes, and in different endo- and epithelial tissues (Wulff et al., 2007). Recent findings suggest that IK channels are also expressed in central neurons (Turner et al., 2015), where these channels reduce summation of excitatory postsynaptic potentials (EPSPs) in cerebellar Purkinje neurons (Engbers et al., 2012). Moreover, they contribute to the sAHP and they reduce excitability in CA1 pyramidal cells (King et al., 2015). In myenteric neurons an IK-sensitive sAHP that was blocked by TRAM-34 has also been reported (Nguyen et al., 2007).

BK channels, originally cloned and expressed from Drosophila (dSlo) (Atkinson et al., 1991; Adelman et al., 1992), consist of 6 transmembrane domains (S1-S6). They comprise a pore-forming P-loop, a voltage-sensing domain similar to other Kv channels, and an additional transmembrane segment (S0) for coupling with auxiliary β-subunits (Jan & Jan, 1997; Meera et al., 1997). So far, 4 different β-subunits (β1-4) have been identified in mammals which show different expression and modulate kinetics, Ca²⁺ sensitivity, and pharmacology of BK α-subunits (Orio et al., 2002). For example, β4 is expressed mainly in the brain, slows the activation kinetics, increases the Ca²⁺-sensitivity, shifts the activation threshold of BK current to more negative potentials, and makes the BK α-subunit resistant to the BK channel blockers charybdotoxin and iberiotoxin (Meera et al., 2000; Behrens et al., 2000). At the frog neuromuscular junction, BK channels regulate transmitter release through modulation of presynaptic Ca²⁺ influx (Robitaille & Charlton, 1992). Later, BK channels were also found at pre-synaptic sites and somata in the rat brain (Knaus et al., 1996). In rat CA1 pyramidal cells, BK channels contribute to the somatic spike repolarization, and mediate the fAHP (Storm, 1987; Shao et al., 1999). At hippocampal pre-synaptic terminals, BK channels do not affect waveforms and transmitter release under basal conditions, but they can repolarize pre-synaptic spikes and thereby reduce transmitter release when other Kv channels are blocked (Hu et al., 2001; Alle et al., 2011).

Investigations of Ca²⁺-activated K⁺ channels are presented in Paper III and Paper IV.
Dorso-ventral differences in the hippocampus

The hippocampus is a medial temporal lobe structure which is found in different mammalian species, including rat, monkey and human. In rat and other rodents the hippocampus is orientated along the dorso-ventral (septo-temporal) axis, which corresponds to a posterior-anterior orientation in primates. The hippocampus is critically involved in memory formation, initially described in patient H.M. (Henry Molaison), who suffered from severe memory loss after bilateral hippocampal resection (Scoville & Milner, 1957).

Subsequent studies in rats found a role of the hippocampus in spatial memory, based on the discoveries of place cells (O'Keefe & Dostrovsky, 1971) and the impairment of spatial memory after hippocampal lesions (Morris et al., 1982). In addition, the hippocampus is also involved in contextual fear memory (Kim & Fanselow, 1992).

Later work suggested that the hippocampus differs in its functions, connections, and gene expression along the dorso-ventral axis (Moser & Moser, 1998; Fanselow & Dong, 2010; Strange et al., 2014). For example, lesions in dorsal but not ventral hippocampus of rats impair spatial memory in a Morris water maze test (Moser et al., 1993). Conversely, ventral hippocampal lesions reduce defensive fear response (Kjelstrup et al., 2002) and affect feeding behavior (Bannerman et al., 2002). Moreover, stimulation of the ventral but not the dorsal hippocampus increases locomotion through activation of the nucleus accumbens and the dopaminergic pathways of the mesolimbic system (Wu & Brudzynski, 1995; Zhang et al., 2002; Peleg-Raibstein & Feldon, 2006). The activation of the dopaminergic reward-pathways by the ventral hippocampus may be of particular importance for early goal-oriented learning during spatial navigation (Ruediger et al., 2012).

Anatomical data suggest that cortical areas involved in spatial navigation (retrosplenial cortex) are connected to the dorsal hippocampus via dorsal parts of the entorhinal cortex, whereas areas involved in emotional processing (infralimbic and prelimbic cortices) are connected to the more ventral parts of entorhinal cortex and hippocampus (Jones & Witter, 2007; Strange et al., 2014). The projections from hippocampus to subcortical areas are also different along the hippocampal dorso-ventral axis. The dorsal hippocampus preferentially targets small parts of the dorsal (rostral) lateral septum, the mammillary body and the anterior hypothalamic nucleus, whereas the ventral hippocampus targets the more ventral parts of lateral septum, the medial preoptic nucleus, the hypothalamic periventricular zone, the ventromedial hypothalamic nucleus and the medial parts of nucleus accumbens and amygdala (Groenewegen et al., 1987; Risold & Swanson, 1996; Risold & Swanson, 1997; Kishi et al., 2006; Strange et al., 2014).

Gene expression data indicate multiple molecular domains along the hippocampal dorso-ventral axis with sharply defined borders (Dong et al., 2009; Fanselow & Dong, 2010). The individual domains are defined by an overlap of many genes with common expression boundaries. Moreover, the number of domains may vary between different hippocampal subfields. In CA1, for example, a dorsal, an intermediate and a ventral domain have been described (Dong et al., 2009), whereas in CA3, nine expression domains have been found along its dorso-ventral axis (Thompson et al., 2008). In these studies a large number of genes encode for proteins involved in cell adhesion, axon guidance, neuropeptides, neurotransmitter receptors, ion channels and transcription factors.
Electrophysiological recordings show that hippocampal pyramidal neurons have spatially selective firing fields (O'Keefe & Dostrovsky, 1971), and the field size increases almost linearly along the dorso-ventral axis (Jung et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008; Royer et al., 2010). In parallel, CA1 pyramidal neurons also differ in synaptic plasticity (Papatheodoropoulos & Kostopoulos, 2000; Maggio & Segal, 2007) and in their intrinsic properties (Dougherty et al., 2012; Marcelin et al., 2012a; Marcelin et al., 2012b; Dougherty et al., 2013). These findings include dorso-ventral differences in the ability to induce long-term potentiation (LTP) (Papatheodoropoulos & Kostopoulos, 2000) and to modulate LTP by corticosteroids (Maggio & Segal, 2007). Furthermore, dorsal and ventral CA1 pyramidal cells differ in their intrinsic excitability (Dougherty et al., 2012), expression of HCN genes (encoding for hyperpolarization-activated cyclic nucleotide gated (HCN) channels), HCN-dependent $h$-current ($I_h$) and $I_h$-dependent functions (Marcelin et al., 2012a; Dougherty et al., 2013). In addition, Marcelin et al., 2012b found dorso-ventral differences in Kv4.2 protein levels and Kv4.2 mediated current. The properties and impact of other $K^+$-dependent membrane currents along the hippocampal dorso-ventral axis remain unknown. Given the importance of $I_M$ in regulating excitability, spike rate adaptation and other important functions in central neurons, we investigated its role in CA1 pyramidal neurons along the dorso-ventral axis (see Paper I). It’s not known whether other hippocampal cell types also differ in their intrinsic properties. In Paper III, we investigated the basic electrical properties of dentate granule cells along the dorso-ventral axis.
AIMS

The aims of this Ph.D.-project were:

1) Test whether dorsal and ventral CA1 pyramidal cells differ with respect to Kv7/M-current ($I_M$) and $I_M$-related functions, including resonance, temporal summation, and excitability (Paper I).

2) Investigate the electrical properties and functions of Kv2 channels in layer II stellate cells in medial entorhinal cortex (Paper II).

3) Test whether dentate granule cells differ in their intrinsic properties along the hippocampal dorso-ventral axis (Paper III).

4) Test whether IK1 channels (SK4; KCNN; intermediate conductance Ca$^{2+}$-activated K$^+$ channels) underlie the sAHP and control repetitive firing in CA1 pyramidal cells (Paper IV).
METHODS

The majority of our experiments were performed with patch-clamp recordings from neurons in acute slices from rat or mouse brains.

The patch-clamp method allows measurements of whole-cell or single-channel currents flowing across cellular membranes through ion channels. Measurements of small currents in the picoampere range (10^{-12} A) requires a low-noise recording technique, achieved by a high-resistance seal (“gigaseal, > 10^9 Ω) between the cellular membrane and the pipette tip. The method can be used to measure either the current flow, by controlling the voltage of a cell membrane (voltage clamp), or to measure the voltage response, by manipulating the current through the pipette (current clamp). The voltage-clamp method is particularly useful for determining the voltage dependence and kinetics of membrane currents, whereas current clamp is commonly used for studying physiological voltage responses such as action potentials and after-hyperpolarizations (AHPs).

The patch-clamp technique allows different recording configurations, including cell-attached, inside-out, outside-out and whole-cell recordings, each with its own advantages and disadvantages. Cell-attached recordings are noninvasive and leave the intracellular environment intact, which is especially important if the channel gating is under metabolic control and effected by intracellular second messengers. A disadvantage is that the membrane potential can’t be measured directly, and the ionic composition inside the cell cannot be changed.

The inside-out patch is achieved by establishing a cell-attached configuration followed by retraction of the pipette and excision of the patch. In this way, the cytosolic side of the membrane is exposed to the extracellular medium, which allows systematic investigation of intracellular messengers involved in ion channel gating. The main disadvantage is that cytosolic factors are lost which may affect ion channel gating. Further, some ion channels are also anchored to the cytoskeleton and may not be retained after patch excision.

The outside-out patch is achieved by first establishing a cell-attached configuration followed by brief suction, i.e. applying negative pressure to the patch pipette, leading to the rupture of the patch membrane and “break-in” to the intracellular environment. After achieving the “whole-cell” configuration the pipette is slowly retracted leading to excision of an “outside-out” patch. This configuration is useful for studying extracellular factors involved in ion channel gating. The disadvantages are similar to those described in inside-out patches, i.e. loss of intracellular messengers and possible retention of ion channels anchored to the cytoskeleton.

A special form of outside-out patch - the nucleated patch- was used in paper II in order to study macroscopic Kv2 current under improved voltage and space clamp conditions. This configuration is achieved in a manner similar to that of the outside-out patch, but additional negative pressure during pipette retraction extracts also the nucleus and larger parts of the cellular membrane. The main advantage of the nucleated patch compared to the conventional outside-out patch is an improved signal-to-noise ratio since the larger membrane surface results in larger current and increased stability during longer recording periods.
The whole-cell recording configuration was used in the majority of the experiments. The advantage of this configuration is to measure the summed response of all ion channels that determine the intrinsic properties of the cell. In contrast, the whole-cell configuration also causes loss of cytosolic molecules through the dialysis with the intracellular pipette solution, and this may affect the cellular responses.

To enable stable whole-cell patch clamp recordings of visually identified neurons under controlled, standardized conditions, we chose an acute brain slice preparation.

Before slice preparation, rats and mice were deeply anaesthetized with Suprane (desflurane) followed by rapid decapitation. The brain was quickly removed (<1 min) and transferred to a petri-dish, containing an ice-cold (0°C) sucrose-based cutting medium with low Na⁺ concentration, and saturated with 95% O₂/5% CO₂ for better tissue preservation (see Methods in the papers). Acute slices were cut from hippocampus or entorhinal cortex in sucrose-based artificial cerebrospinal fluid (aCSF) with a vibratome. The slices were immediately transferred to a submerged holding chamber and incubated at 30-32°C for ~30 min. The slices were then stored at room temperature (20-24°C) before being transferred to the recording chamber, containing aCSF (see Methods in the papers). Recordings were done at 34-35°C, which is close to the normal brain temperature of rats and mice in vivo (36-37°C).

Prior to recording, a patch pipette was pulled from borosilicate glass and filled with a intracellular solution containing either potassium methylsulfate, potassium gluconate, or potassium methanesulfonate. The pipette was then positioned close to the surface of the brain slice, viewed with an upright microscope with infrared-differential interference contrast (IR-DIC) optics. While entering the recording chamber, positive pressure was applied to the inside of the pipette, which avoids clogging of the pipette tip with dirt and tissue debris. The seal formation between pipette and cell membrane was monitored by observing current responses evoked by small negative voltage pulses. Approaching the cell membrane usually decreased the current response, due to an increase in seal resistance. The release of positive pressure was followed by gentle suction led to a further increase in seal resistance until a “gigaseal” was reached. After cancelling the remaining capacitive currents of the pipette, the current trace appeared flat. Whole-cell recordings were finally achieved by applying brief negative pressure to the inside of the pipette, causing rupture of the cell membrane below the patch and the appearance of a cellular capacitive transient.
RESULTS AND DISCUSSION

Paper I

It has been suggested that the hippocampus differs in its functions, connections and gene expression along the dorso-ventral (D-V) axis (Moser & Moser, 1998; Bannerman et al., 2004; Fanselow & Dong, 2010; Strange et al., 2014).

In this study we aimed to determine the role of Kv7/M-channels in regulating the intrinsic properties of dorsal and ventral CA1 pyramidal cells in young, 3-4 week old rats.

Our voltage-clamp recordings indicated that dorsal CA1 pyramidal cells exhibit a larger Kv7/M-current, $I_{M}$, and a hyperpolarizing shift in its activation range compared to ventral pyramidal cells. Using current-clamp recordings, we found that $I_{M}$ had a stronger impact on voltage responses in dorsal than ventral pyramidal cells.

We found that temporal summation was similar in dorsal and ventral CA1 pyramidal cells under normal conditions but stronger in dorsal than in ventral cells when Kv7/M-channels were blocked with XE991. Thus, Kv7/M-channels seem to “normalize” temporal summation under normal conditions. However, this finding also suggest that suppression of Kv7/M-current by acetylcholine or other cholinergic agonists (Brown & Adams, 1980; Halliwell & Adams, 1982; Storm, 1989) may enhance temporal summation and facilitate synaptic integration, preferably in the dorsal compared to the ventral pyramidal cells.

We hypothesized that a stronger Kv7/M-current in dorsal vs. ventral cells could also affect $I_{M}$-dependent resonance (M-resonance) in the theta frequency range (Hu et al., 2002; Hu et al., 2009). Indeed, we found that dorsal cells showed prominent M-resonance at subthreshold potentials, while M-resonance was weaker and rarely found in ventral cells. However, application of the selective Kv7/M-channel opener retigabine strongly increased M-resonance in ventral cells. This suggested that functional Kv7/M-channels are present in the ventral cell population, but normally weakly activated within the subthreshold voltage range.

Interestingly, it has been suggested that differences in resonance properties along the hippocampal D-V axis may contribute to the D-V difference in theta rhythmicity (Royer et al., 2010). In addition, previous studies reported D-V differences in HCN-dependent resonance and temporal summation in CA1 pyramidal cells (Marcelin et al., 2012a; Marcelin et al., 2012b; Dougherty et al., 2013). This raised the question whether D-V differences in $I_{M}$, $I_{h}$ or other intrinsic membrane currents can account for differences in place field size along the hippocampal D-V axis (Jung et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008; Royer et al., 2010). Although several factors may contribute to this D-V gradient, experiments in HCN1 knock-out mice showed larger place fields in hippocampal pyramidal cells (Hussaini et al., 2011) presumably reflecting the relevance of intrinsic currents in vivo.

We also found that Kv7/M-channels have a stronger impact on spike threshold, the Kv7-dependent mAHP, excitability and spike adaptation in dorsal than in ventral pyramidal cells. Moreover, the f/I curves, as probed with somatic injection of depolarizing current pulses, were steeper (i.e. the excitability was more sensitive to the input) in ventral compared to dorsal cells as previously found by Dougherty et al., (2012). In parallel, ventral cells exhibited also weaker spike frequency adaptation than dorsal cells. However, when Kv7/M-channels were
blocked, the D-V difference in $f/I$ curves and spike adaptation were abolished, suggesting that they were directly caused by D-V differences of $I_M$. Notably, it has been reported that the ventral hippocampus is more susceptible to epileptiform activity than the dorsal hippocampus (Bragdon et al., 1986; Derchansky et al., 2004). This may be partially due to reduced Kv7/M-channel activity in ventral compared to dorsal pyramidal cells.

**Paper II**

The roles of different potassium channels in regulating excitability and spike properties in the medial entorhinal cortex (mEC) have so far been characterized only to a very limited extent. In paper II we investigated the electrical properties of Kv2 channels in layer II stellate cells using the recently identified Kv2 channel blocker Guangxitoxin-1E (GTx-1E).

First, we performed voltage-clamp recordings from nucleated patches and found that 100 nM GTx-1E inhibited a delayed-rectifier current ($I_K$), but had no effects on the transient A-type current ($I_A$), as previously found (Liu & Bean, 2014).

In current clamp, wash-in of 100 nM GTx increased burst firing and excitability when starting from moderate spike rates (10 spikes/s). In contrast, at higher spike rates (70 spikes/s), wash-in of GTx decreased maintained firing and spike amplitudes and depolarized interspike-intervals following the first spike. With lower doses of GTx (10 and 30 nM), we found that block of Kv2 channels increased burst-firing and excitability by increasing the after-depolarization (ADP), and by reducing the fast and medium after-hyperpolarizations (fAHPs, mAHPs) during repetitive firing in LII SCs.

Previously, it has been shown that block of Kv7/M-type K$^+$ current with linopirdine has only weak effects on ADP and firing patterns in LII SCs (Yoshida & Alonso, 2007). A recent study reported that block of Kv7/M-channels with XE991 increased the excitability of LII SCs (Nigro et al., 2014), but did not increase the ADP and burst-firing as found in pyramidal cells of the hippocampus and mEC (Yue & Yaari, 2004; Gu et al., 2005; Yoshida & Alonso, 2007). Thus, our present results combined with those of previous studies suggest that Kv2 channels in LII SCs have a strong impact on ADPs and burst-firing in contrast to the impact of Kv7/M-channels. It has been shown that, postsynaptic burst-firing paired with presynaptic activity can lead to induction of long-term potentiation (LTP) (Magee & Johnston, 1997; Thomas et al., 1998). It remains to be determined whether Kv2 channels can regulate LTP induction by modulating the ADP and burst-firing in LII SCs.

We also found that block of Kv2 channels with GTx caused an increase in spike broadening during repetitive firing, but had no effect on the first spike. This indicates that Kv2 channels contribute directly to late spike repolarization. However, this effect may also be indirectly due to more depolarized inter-spike trajectories after Kv2 blockade, followed by incomplete recovery of inactivation of voltage-gated Na$^+$ channels, thereby reducing spike amplitudes and activation of other repolarizing K$^+$ channels.

The block of Kv2 channels and its effects on inter-spike trajectories, spike repolarization and Na$^+$ channel availability are also probably the main reason for an observed reduction in maintained firing during higher spike rates, as previously suggested in other cell types (Du et al., 2000; Tong et al., 2013; Guan et al., 2013; Liu & Bean, 2014).
**Paper III**

In this study, we compared the sAHP amplitude, the excitability and the firing pattern of dentate granule cells (DGCs) at different locations along the hippocampal dorso-ventral axis in juvenile and adult rats, and adult mice. In all age groups tested, the sAHP amplitude, and spike frequency adaptation were more prominent, i.e. the excitability was lower, in the dorsal than in the ventro-intermediate DGCs. Our results from current clamp experiments were confirmed when we used different intracellular solutions and slicing procedures. In addition, morphological reconstructions of previously recorded DGCs indicated that the dorso-ventral differences in sAHP amplitude, spike frequency adaptation and excitability were not correlated with variations in dendritic and axonal lengths. In addition, using whole-cell voltage clamp recordings, we found that the current underlying the sAHP ($I_{sAHP}$) showed a bigger peak amplitude and slower kinetics in dorsal than ventral DGCs.

It has been suggested that different channel types may underlie the $I_{sAHP}$ of DGCs. One study found that knock-out of Kv7.2 or Kv7.3 in mice decreased the $I_{sAHP}$ peak amplitudes in DGCs (Tzingounis & Nicoll, 2008). Additionally, we have previously shown in CA1 pyramidal cells that the $I_M$ mediated by Kv7/M-channels causes dorso-ventral differences in several $I_M$-dependent properties including excitability and spike frequency adaptation (paper I). Therefore, it is relevant to ask whether $I_M$ may also contribute to dorso-ventral differences in $I_{sAHP}$ in the DGCs. A recent study showed that DGCs with short axons (less than 10 µM) show reduced or absent $I_M$ (Martinello et al., 2015). Conversely, we have shown that dorso-ventral differences in sAHP amplitude, spike rate adaptation and excitability were not correlated to variations in axonal (and dendritic) length, even in DGCs with very short axons, i.e. less than 10 µM. Further, we observed D-V differences in $I_{sAHP}$ amplitude and kinetics in presence of 5 mM TEA, which partially blocks Kv7/M-channels (Storm, 1990; Battefeld et al., 2014). Hence, a major contribution of $I_M$ to dorso-ventral differences in $I_{sAHP}$ in DGCs seems unlikely. Tanner et al., 2011 found that $K_{ATP}$ channels may contribute to sAHP following a spike train in DGCs. They concluded that the $K_{ATP}$ channel opening was due to ATP depletion in response to spike firing. In our present study, we observed D-V differences in $I_{sAHP}$ amplitude and kinetics while $Na^+$ channels and spike firing were blocked with TTX. Thus, although some ATP depletion might conceivably also be caused by the calcium influx that triggered the sAHP, our findings indirectly suggest that $K_{ATP}$ channels are not the main cause for the observed dorso-ventral differences in $I_{sAHP}$ in DGCs.

Future experiments will be needed to identify the mechanism underlying the dorso-ventral differences in sAHP, spike accommodation and excitability in DGCs. The reduced sAHP amplitude and higher excitability in ventral DGCs may contribute to an early onset of epileptiform activity in the ventral hippocampus in models of temporal lobe epilepsy (Derchansky et al., 2004; Toyoda et al., 2013).

**Paper IV**

Our aim was to test whether intermediate conductance $Ca^{2+}$ activated $K^+$ channels (IK1) underlie the slow after-hyperpolarization, sAHP, in CA1 pyramidal cells, as recently suggested (King et al., 2015).
Using somatic whole-cell voltage clamp, we first recorded a prominent $I_{sAHP}$ tail current in response to depolarizing voltage pulses in CA1 pyramidal cells in rat hippocampal slices. To test whether IK1 channels underlie the $I_{sAHP}$, we bath-applied the potent IK1 blocker TRAM-34 (Wulff et al., 2000) or delivered the drug intracellularly via the patch pipette, but found no effects of TRAM-34 during a time course of 25 minutes. In some experiments, we also applied the cholinergic agonist carbachol after the 25 minutes. Carbachol potently blocked the $I_{sAHP}$, as previously shown (Benardo & Prince, 1982; Cole & Nicoll, 1984; Storm, 1990). In a separate experiment, the $I_{sAHP}$ amplitude was monitored during wash-in of the IK1 blocker charybdotoxin (ChTx) (Ishii et al., 1997). Again, block of IK1 channels had no significant effect on $I_{sAHP}$. To test whether TRAM-34 and ChTx were able to block IK1 channels, both compounds were bath-applied to HEK293 cells transiently expressing IK1 channels which rapidly blocked IK1 currents. Next we investigated the effects of TRAM-34 on AHPs and excitability in CA1 pyramidal cells in current clamp configuration. Similar to our findings in voltage clamp, TRAM-34 had no effects on mAHP/sAHP amplitudes, and did not change excitability in response to depolarizing current pulses. In a different set of experiments we bath-applied a combination of K$^+$ channel blockers, including TRAM-34, XE991 to block Kv7 channels, and apamin to block SK channels, as previously used by King et al. (2015). This mixture of K$^+$ channel blockers suppressed the mAHP but again had no effects on the sAHP. In our study, the effects of TRAM-34 in pyramidal neurons of the basolateral amygdala (BLA) were also measured, but again no effect on the $I_{sAHP}$, excitability, or spike properties were found, in agreement with our findings in CA1 pyramidal neurons. Finally, our experiments in IK1 knock-out mice revealed a prominent $I_{sAHP}$ that seemed indistinguishable from that of wild type mice.

In conclusion, our experimental data indicate that IK1 channels do not contribute to the current underlying the sAHP and have no effects on excitability in pyramidal cells in BLA or CA1. Our present data are inconsistent with the recent study that reported a reduction in $I_{sAHP}$ and sAHP in CA1 pyramidal cells after block of IK1 channels with TRAM-34 (King et al., 2015). This discrepancy may be due to a combination of several factors: 1) run-down of $I_{sAHP}$ during whole-cell recordings has previously been observed and cannot be excluded in the absence of time plots. 2) The Ca$^{2+}$ influx and, hence, $I_{sAHP}$, may differ between synaptic stimulation (King et al., 2015) and injection of somatic current pulses, due to recruitment of e.g. different Ca$^{2+}$ sources. This could affect activation of IK1 channels depending on its subcellular localization.

Further, it has been proposed that the sAHP is mediated by multiple classes of K$^+$ channels which are gated by diffusible cytosolic Ca$^{2+}$ sensors such as hippocalcin or neurocalcin combined with changes in membrane phosphatidylinositol 4,5-bisphosphate levels (Andrade et al., 2012). More experiments will be needed to unequivocally identify the molecular components underlying the $I_{sAHP}$, using gene manipulations of different pore-forming and accessory Kv subunits, together with cloned components and specific pharmacological tools.


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