FUNCTIONAL ROLES OF SK2 CHANNELS IN AREA CA1 OF THE HIPPOCAMPUS

by

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CERTIFICATE OF APPROVAL

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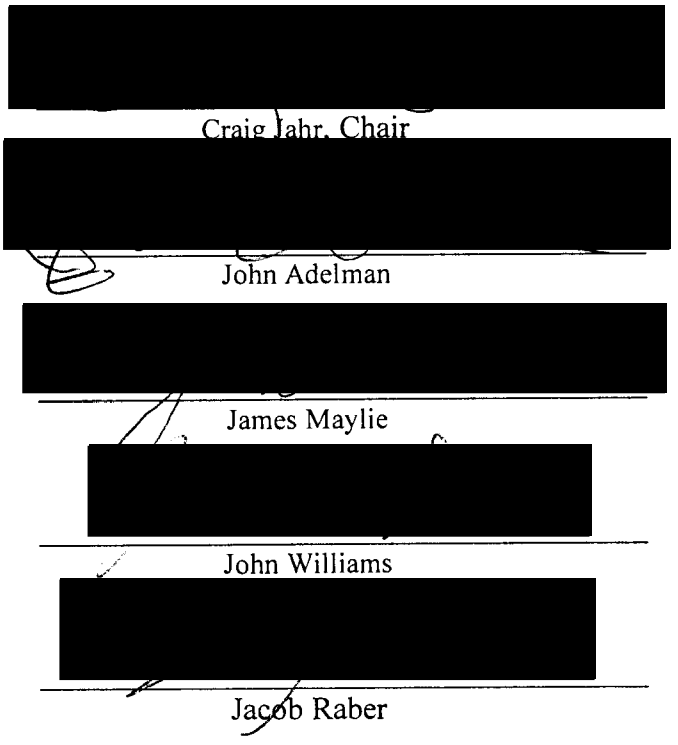

To Jürgen and my family

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic
BK	large-conductance potassium channel
Ca^{2+}	calcium
EPSP	excitatory postsynaptic potential
IPSP	inhibitory postsynaptic potential
K^+	potassium
LTD	long-term depression
LTP	long-term potentiation
Na^+	sodium
NMDA	N-methyl-D-aspartate
SK	small-conductance potassium channel

ABSTRACT

Ca^{2+}-activated small-conductance K^+ (SK) channels play a fundamentally important role in all excitable cells. They are potassium selective, voltage-insensitive and are activated by increases in the levels of intracellular Ca^{2+} such as occur during an action potential (Zhang et al., 1995; Köhler et al., 1996; Sah et al., 1991; Lorenzon et al., 1992). As the action potential decays, the membrane potential is repolarized, and internal Ca^{2+} levels rise, evoking a biphasic afterhyperpolarization. The initial faster phase is thought to be due to the activation of large-conductance voltage- and Ca^{2+}-activated K^+ (BK) channels, while the slower phase is thought to be due to the activation of SK channels, which are gated solely by intracellular Ca^{2+} ions (Storm, 1990; Sah, 2002). While it is clear that SK channels mediate an apamin-sensitive current in CA1 pyramidal neurons, there has been controversy as to whether or not SK channels contribute to the afterhyperpolarization (Bond et al., 2004; Gu et al., 2005).

Increases in the intracellular Ca^{2+} concentration activate SK channels, leading to hyperpolarization of the membrane potential, which in turn reduces the Ca^{2+} inflow into the cell. This feedback mechanism is ideally suited to regulate the spatiotemporal occurrence of Ca^{2+} transients: as SK channels activate, they extrude potassium from the cell, moving the membrane to more negative potentials. The recovery of the Ca^{2+} signal following an action potential is slow, permitting SK channels to generate a long-lasting hyperpolarization with a time course that reflects the decay of intracellular Ca^{2+}. Thus, activation of SK channels causes membrane hyperpolarization, which inhibits cell firing.

The first aim of this thesis was to assess and quantify the contribution of SK channels to the afterhyperpolarization in CA1 pyramidal neurons; whole-cell current-clamp recordings in mouse hippocampal pyramidal cells were performed. To characterize the afterhyperpolarization, bursts of action potentials were elicited by either injection of single current pulses or by a constant depolarizing 400ms current pulse into the soma at the normal resting potential. We find that under these conditions the afterhyperpolarization, elicited after bursts of action potentials of varying number (5-50 action potentials) and frequency (5-50 Hz), is reduced upon application of the selective SK channel blocker apamin. In contrast to other reports, we find that the M-channel blocker XE991 has little or no effect on the afterhyperpolarization.

These results provide evidence that SK channels are activated by bursts of action potentials and contribute to the generation of the afterhyperpolarization in CA1 pyramidal neurons.

Excitatory synaptic transmission in the mammalian central nervous system is mediated by glutamate, which activates two types of ionotropic receptors: AMPA receptors and NMDA receptors. At most synapses, basal synaptic transmission is largely mediated by activation of AMPA receptors. NMDA receptors, which are coexpressed at most synapses, make a minimal contribution to basal transmission but are recruited when cells are depolarized. Glutamatergic synapses are formed onto dendritic spines, and activation of NMDA receptors leads to a compartmentalized rise in spine Ca^{2+} (Köster and Sakmann, 1998; Kovalchuk et al., 2000). Recently it has become clear that synaptic integration is critically regulated by the concomitant activation of numerous voltage-

gated ion channels in the dendritic membrane (Johnston et al, 1996; Magee et al., 1998). As activation of synaptic NMDA receptors has been shown to lead to localized rises in spine Ca^{2+}, it raises the possibility that channels present in the postsynaptic membrane could be modulated by this rise in intracellular Ca^{2+}. Ca^{2+}-activated small-conductance potassium channels are also known to be present in dendrites where they can shunt synaptic potentials (Cai et al., 2004). Other recent reports have shown that in addition to their activation during action potential discharge, SK channels can be activated by Ca^{2+} rises from other sources, such as release from intracellular stores or dendritic activation of NMDA receptors (Grillner et al., 2001). Furthermore, blockade of SK channels with apamin has been reported to enhance LTP of synaptic transmission in the hippocampus (Behnisch and Reymann, 1998; Stackmann et al., 2002).

It will be shown in the second aim of this thesis that in hippocampal pyramidal neurons, SK channels are activated by Ca^{2+} influx through synaptically activated NMDA receptors, resulting in attenuation of the synaptic potential. Thus, blockade of SK channels by apamin potentiates fast glutamatergic synaptic potentials.

Together, the results from these studies provide evidence that separate populations of SK2 channels in CA1 neurons occupy discrete subcellular locations where they couple with different Ca^{2+} sources to serve distinct physiological roles.

I. INTRODUCTION

I.1. The hippocampus

I.1.1. Overview

The hippocampus is one of the most thoroughly studied areas of the mammalian central nervous system. There are two main reasons for this. First, it has a distinctive and readily identifiable structure at both the gross and the histological level - it is the histology of the hippocampus that makes it so seductive to neuroscientists. The hippocampus is beautifully laminated; both the neuronal cell bodies and the zones of connectivity are arranged in orderly layers. This highly laminar nature of the hippocampus lends it to neuroanatomical and electrophysiological studies.

A second reason for the interest in the hippocampus is that since the early 1950s, it has been recognized to play a fundamental role in certain forms of learning and memory. The most striking example of a role for the hippocampus in learning and memory is the patient H.M., who was treated for intractable epilepsy by the removal of both hippocampi. Following surgery H.M. had a profound loss of memory for new events although his motor skills and ability to recall events prior to surgery were relatively intact (Scoville and Milner, 1957). These studies have forged a strong theoretical link between the hippocampus and related medial temporal lobe structures with certain forms of memory. In addition, hippocampal lesions selectively impair memory of recently acquired information, while leaving more remote memories intact, suggestive of a role for the hippocampus in memory consolidation. The hippocampal formation is also of interest because of its high seizure susceptibility – it has the lowest seizure threshold of

any brain region. Furthermore, portions of the hippocampal formation appear to be prime targets for the pathology associated with Alzheimer's disease, and the hippocampus is very vulnerable to the effects of ischemia and anoxia.

Persistent changes in neural circuits induced by electrical activity are essential for learning and memory functions of the mature brain (Turrigiano, 1999). Based upon lesion studies and diseases that affect the morphology and function of the hippocampus, it is widely believed that the integration that occurs during hippocampal processing is essential for the formation of long-term memory. It is the hippocampus, in which increased probability of neuronal spiking occurs in parallel with tetanus-induced long-term potentiation (LTP) (Oh et al., 2003). Part of this apparent increase in excitability can be accounted for by postsynaptic changes of active conductances in the dendrite.

I.1.2. Anatomy

The *in vitro* hippocampal slice preparation permits evaluation of the electrophysiological properties of hippocampal pyramidal neurons independent of inputs from other brain regions to study changes localized to the hippocampus.

In the mouse, the hippocampus is located dorsally, above the thalamus. During evolution of the temporal lobe, these structures migrated into the temporal lobe, leaving behind a fiber pathway (the *fornix*), which is located above the thalamus.

The *alveus* is a thin layer of afferent and efferent fibers that wraps around the entire outer surface of the hippocampus. Just within the *alveus* lies the *stratum oriens*, which contains the basal dendrites of the pyramidal cell neurons, whose cell bodies are located in the *stratum pyramidale*. The apical dendrites are contained within the

remaining two layers, the *stratum radiatum* (containing proximal dendrites) and the *stratum lacunosum-moleculare* (containing distal dendrites). These layers of the hippocampus are also curved in structure, forming a "C" shape. At the CA3 end of the "C" lays the dentate gyrus, which forms an interlocking "U" shape around the layers of CA3. Because of this curved shape, the hippocampus was named for its resemblance to the sea horse (Greek: "hippo" – horse, "kampos" – sea monster).

The hippocampal formation is composed of three distinct regions: the hippocampus proper (Ammon's horn), the dentate gyrus and the subicular region. The hippocampus proper consists of a three-layered cortical area forming a large mass, which actually intrudes into the ventricular space of the inferior horn of the lateral ventricle. This cortical region has been divided into a number of subportions (CA1-4). The dentate gyrus is a phylogenetically older cortical area, consisting of only three layers. During the formation discussed above, the leading edge of the cortex detaches itself and becomes the dentate gyrus. Parts of it remain visible at the surface of the brain.

The hippocampus receives input from many lower brain regions including the medial septal nuclei, the ventral tegmental area, the raphe nuclei, and the locus coeruleus. Cortical projections derive from the entorhinal cortex and innervate the granule cells of the dentate gyrus via the perforant pathway. The granule cells project to the CA3 pyramidal cells, which in turn project via collaterals to the opposite hippocampus or to the CA1 pyramidal cells: the Schäffer collateral and perforant path inputs arrive at very different locations in the dendrites of CA1 pyramidal neurons. The perforant path terminates at thin and distal dendrites in *stratum lacunosum-moleculare* more than 300μm from the soma. In contrast, the Schäffer collaterals arrive more proximally in the

stratum radiatum. CA1 pyramidal cells project to the subiculum, which relays to the entorhinal cortex, thus completing the circuit. CA1 cells also project to the lateral septal nuclei.

The cell bodies of the hippocampal pyramidal neurons are arranged, three to six cells deep, in an orderly layer called the *pyramidal cell layer*. These neurons have elaborate dendritic trees extending perpendicularly to the cell layer in both directions and are thus considered to be multipolar neurons but more typically called pyramidal cells. The apical dendrites are longer than the basal dendrites and extend from the apex of the pyramidal cell body toward the center of the hippocampus, i.e. toward the dentate gyrus. The dendrites of the pyramidal neurons are covered with spines onto which most excitatory synapses terminate. Megias et al. (2001) estimated that a typical CA1 pyramidal neuron may have as many as 30.000 excitatory inputs and 1.700 inhibitory inputs. The proximal apical dendritic shaft is heavily innervated by inhibitory synapses.

The dendritic trees with their spines participate extensively in the spatial and temporal integration of synaptic input. Rather than acting as passive cables, dendrites serve as sophisticated signal processors. The dendrites of hippocampal pyramidal neurons possess a wide variety of voltage-gated ion channels that are expressed throughout the apical and basal dendrites (for a detailed review see Magee et al., 1998). Some types of channel are uniformly distributed in the dendrites while others are found to have very non-uniform densities (Johnston et al., 1996; Magee et al., 1998). Non-uniformities in channel distributions make dendritic electrical properties markedly different from those at the soma. For example, TTX-sensitive Na^+ channels have a uniform density from the soma to at least the first 350μm of the apical dendrites, but their biophysical properties

differ between those found in the soma and distal dendrites in several important ways (Colbert et al., 1997).

Potassium channels located in the dendrites of hippocampal CA1 pyramidal neurons control the shape and amplitude of back-propagating action potentials, the amplitude of excitatory postsynaptic potentials and dendritic excitability (for a review see Magee et al., 1998). For example, the influence of a fast, Ca^{2+}-activated potassium current on action potential repolarization is progressively reduced in the first 150μm of the apical dendrites, so that action potentials recorded farther than 200μm from the soma have no fast afterhyperpolarization and are wider than those in the soma (Johnston et al., 2000). The lack of a fast Ca^{2+}-dependent K^+ current in distal dendrites is at least one factor responsible for significantly broader dendritic action potentials, which may provide an effective time window for unblocking NMDA receptors and allowing Ca^{2+} influx during the induction of processes like long-term potentiation or depression.

I.1.3. Synaptic transmission at the Schäffer collateral-CA1 synapse

During excitatory synaptic transmission, the neurotransmitter glutamate binds to two different subtypes of receptor that are often, but not always, colocalized on individual dendritic spines, the small (~1 μm^3) outgrowths from the dendritic shaft that are the postsynaptic site of synaptic contacts.

The first is the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor, which has a channel permeable to monovalent cations (Na^+ and K^+) and which provides the majority of inward current for generating synaptic responses when the cell is close to its resting membrane potential. While Na^+ through AMPA receptors mediates the

fast component of the excitatory postsynaptic potential (EPSP), Ca^{2+} through the second subtype of glutamate receptors, the *N*-methyl-D-aspartate (NMDA) receptors, contributes a slower-rising and slower-decaying component to the EPSP (Forsythe and Westbrook, 1988).

Activation of the NMDA receptor and concurrent depolarization results in the development of a relatively slow-rising, long-lasting current mediated primarily by the influx of Ca^{2+}. NMDA receptor-mediated Ca^{2+} transients following release of a single vesicle of glutamate reach ~1-10 μM in spines held at resting and depolarized potentials (Sabatini et al, 2002). At a single CNS synapse, NMDA receptors usually coexist with either AMPA or kainate receptors and are thought to be involved in amplification of the glutamate signal, although examples of primarily NMDA receptor-mediated synaptic responses have been reported (Isaac, 2003). At resting potentials, Mg^{2+} normally blocks NMDA channels and there must be sufficient concurrent depolarization of the postsynaptic neuronal membrane (to about -30 mV) before the Mg^{2+} block is relieved and the NMDA receptor channel can contribute to the electrical response of the cell (Mayer and Westbrook, 1985). The level of concurrent depolarization depends on AMPA/kainate activation and/or other modulatory postsynaptic signals controlling depolarization. It has been shown though that NMDA receptors open relatively readily (~15% of peak conductance) at resting membrane potentials even in the presence of Mg^{2+} and their voltage dependence is relatively weak near resting potentials (Nimchinsky et al., 2002).

The voltage-dependent properties of NMDA-mediated Ca^{2+}-current provides the capacity for Hebbian-type plasticity at synapses where NMDA receptors are located. Repetitive or concurrent activation can depolarize the postsynaptic cell to a level at which

Mg^{2+} block of NMDA-mediated current is relieved and where these channels begin to contribute additional currents to the postsynaptic response. Furthermore, the influx of Ca^{2+} through this channel initiates long-term synaptic and cellular modification - any manipulation that influences the magnitude or dynamics of Ca^{2+} increase within dendritic spines may therefore profoundly influence the form of the resulting synaptic plasticity. The depolarization-dependent removal of Mg^{2+} block of NMDA receptors allows the channel to serve as a molecular detector for the coincidence of presynaptic activation and postsynaptic depolarization. Thus, NMDA receptor channels play a key role in plastic events at the synaptic, cellular, and behavioral level.

Other neurotransmitter receptors are present at hippocampal synapses and act to regulate excitability. The extensive inhibitory network within the hippocampus relies on both $GABA_A$ and $GABA_B$ receptors, which regulate excitability through Cl^- conductance and K^+ channel modulation. Nicotinic acetylcholine receptors are located presynaptically and modulate neurotransmitter release from both excitatory and inhibitory neurons (Wonnacott, 1997). Muscarinic acetylcholine receptors are located both pre- and postsynaptically (Levey, 1996); postsynaptic muscarinic acetycholine receptors modulate K^+ channel conductances and the slow component of the afterhyperpolarization following action potentials (Velumian et al., 1999). In addition, serotonin receptors regulate GABAergic inhibition in CA1 neurons (Turner et al., 2004).

I.1.4. Synaptic plasticity

Synaptic plasticity is a process in which synapses change their efficacy as a consequence of their previous activity. Activity-dependent synaptic plasticity in the

hippocampus is vital to the formation of synaptic connections during development, but is also thought to underlie learning and memory processing throughout adulthood (Huang et al., 1992; Turrigiano 1999).

Perhaps the most quoted idea in the history of neuroscience is the synaptic modification postulate proposed by the Canadian psychologist Donald Hebb in his 1949 book, *The Organization of Behavior*. Formulated to explain how memories could be stored as alterations in synaptic strength, it reads:

> *When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B is increased.*

It was not until 1973, when Bliss and Lomo first reported the phenomenon of long-term potentiation (LTP) in the hippocampus, that a tractable electrophysiological model system emerged that could be used to study use-dependent alteration of synaptic strength in the mammalian brain and that appeared to embody the Hebb postulate.

LTP is a persistent increase in synaptic strength that is typically produced by brief high-frequency stimulation of presynaptic afferents (Bliss and Lomo, 1973) or by pairing presynaptic stimulation with postsynaptic depolarization (Markram et al., 1997). In its most commonly studied form, it requires activation of postsynaptic NMDA receptors and consequent Ca^{2+} influx (Malenka and Nicoll, 1999). Phenomenologically, it appears to obey the Hebb postulate: neither presynaptic activation alone nor postsynaptic activation

alone is sufficient for LTP induction – there is a requirement for association between presynaptic and postsynaptic activity. The properties of the NMDA receptor seem to explain this requirement, as current flux through the NMDA receptor requires both ligation of glutamate (as a consequence of presynaptic activity) and postsynaptic depolarization sufficient to relieve the blockade of the ion channel by external Mg^{2+}, thus implementing Hebb's rule at the synapses. In addition to LTP, several forms of use-dependent synaptic weakening, or long-term depression (LTD), have also been described. At some synapses, LTP and LTD have been shown to reverse each other, thus creating use-dependent bidirectional modification, a computationally useful property (Bear, 1996).

It is well accepted that synaptic activation of NMDA receptors, followed by influx of Ca^{2+} through the NMDA receptor and the consequent rise in cytosolic Ca^{2+} are essential requirements for LTP and LTD: in 1983 Graham Collingridge made the breakthrough discovery that induction of tetanus-induced forms of LTP are blocked by blockade of the NMDA receptor. Collingridge's discovery was that the glutamate analog amino-phosphonovaleric acid (APV), an agent that selectively blocks the NMDA subtype of glutamate receptors, could block LTP induction while leaving basic synaptic transmission intact. This activation of NMDA receptors requires depolarization of the postsynaptic cell, which is usually accomplished experimentally by repetitive tetanic stimulation of synapses or by directly depolarizing the cell while continuing low-frequency synaptic activation (a "pairing protocol") (Gustafsson and Wigstrom, 1991; Nicoll and Malenka, 1995). The consequent rise of intracellular Ca^{2+} is the critical trigger for LTP. This local source of Ca^{2+} within the dendritic spine accounts for the input

specificity of LTP. Associativity occurs because strong activation of one set of synapses depolarizes adjacent regions of the dendritic tree.

The evidence in support of this model is compelling and almost universally agreed upon. Specific NMDA receptor antagonists have minimal effects on basal synaptic transmission but completely block the generation of LTP (Bliss and Collingridge, 1993; Nicoll and Malenka, 1995). Similarly, preventing the rise in postsynaptic Ca^{2+} with Ca^{2+} chelators blocks LTP, whereas directly raising the amount of postsynaptic Ca^{2+} by photolysis of caged Ca^{2+} can mimic LTP (Malenka, 1988; Malenka et al., 1992). Furthermore, imaging studies have demonstrated directly increases in Ca^{2+} within dendritic spines due to NMDA receptor activation (Regehr and Tank, 1990; Yuste and Denk, 1995).

Increases in postsynaptic Ca^{2+} that are NMDA receptor– dependent and that do not reach the threshold for LTP can generate either a short-term potentiation (STP) that decays to baseline over the course of 5 to 20 minutes or long-term depression (LTD), a long-lasting decrease in synaptic strength that may be due to a reversal of the mechanisms underlying LTP (Malenka and Nicoll, 1993, 1999). Thus, any manipulation that influences the magnitude or dynamics of Ca^{2+} increases within dendritic spines may profoundly influence the form of the resulting synaptic plasticity. Although NMDA receptors are the primary source of Ca^{2+} entry into spines, activation of dendritic voltage-dependent Ca^{2+}-channels also substantially raises Ca^{2+} levels and can generate LTP, STP, or LTD. Perhaps because of the distinct subcellular localization of Ca^{2+}-channels, however, the LTP due to activation of Ca^{2+}-channels may use mechanisms distinct from NMDA receptor– dependent LTP (Teyler et al., 1994) and will not be considered further

here. To date, all forms of synaptic plasticity have been found to be NMDA receptor-dependent in area CA1 of the hippocampus.

CA1 neurons are a model system for studying the induction of synaptic plasticity associated with learning and memory; small-conductance Ca^{2+}-activated K^+ (SK) channels have been implicated in these important processes. Apamin application to brain slices facilitates the induction of NMDA receptor-dependent synaptic plasticity, and systemic apamin administration facilitates the acquisition of hippocampal-dependent learning (Stackman et al., 2002). A model to account for the affects of apamin on synaptic plasticity suggests that SK channels in the dendritic spines of CA1 neurons are coupled to NMDA receptors, forming a feedback loop to regulate NMDA-dependent Ca^{2+} influx that is crucial to the induction of synaptic plasticity. Thus, distinct populations of SK channels in CA1 neurons might influence diverse processes.

I.2. Small-conductance Ca^{2+}-activated K^+-channels

I.2.1. Overview

K^+ channels are the largest and most diverse superfamily of ion channels (for a detailed overview of their diversity see Coetzee et al., 1999). They show an exceptional functional diversity, being implicated in regulating membrane excitability of neurons, neurotransmitter release, neural and behavioral plasticity, secretion, and cell proliferation. In addition to regulating cell excitability, the channels themselves can be modulated in a cell-specific manner through second messengers, hormones, and neurotransmitters. The fact that >70 distinct K^+ genes have now been cloned provides a molecular basis for this

diversity. K^+ channels are multimeric membrane proteins that share a high selectivity for K^+. This property is based on a selectivity filter, the structure of which is probably similar in all K^+ channels. Apart from the central pore, K^+ channels vary in membrane topology (2, 4 or 6/7 transmembrane domains), subunit composition and mechanisms of activation and inactivation (Yellen, 2002).

Initial indications that rises in cytosolic Ca^{2+} could change plasmalemmal K^+ permeability in response to changes in intracellular Ca^{2+} concentration were made by Gardos in red blood cells (Gardos, 1958; Lew et al., 1987), where the activation of Ca^{2+}-dependent K^+-channels leads to membrane hyperpolarization and cell shrinkage. In the nervous system, the first Ca^{2+}-activated K^+-currents were described in mollusk neurons (Meech, 1974) and cat spinal motor neurons (Krnjevic and Lisiewicz, 1972). In the early 1980s, Ca^{2+}-activated K^+-currents were shown to underlie the afterhyperpolarization that follows bursts or trains of action potentials in the mammalian hippocampus. It was shown in non-innervated skeletal muscle that the afterhyperpolarization was mediated by Ca^{2+}-activated K^+-channels of small conductance, which were consequently named SK channels. All of these Ca^{2+}-activated K^+-currents can be activated by Ca^{2+} and have a lack of voltage sensitivity, but they differ pharmacologically. Some Ca^{2+}-activated K^+-currents (for example, in cultured skeletal muscle) are blocked by the bee-venom toxin apamin (Blatz and Magleby, 1986), whereas others (for example the slow Ca^{2+}-activated K^+-current sI_{AHP} in hippocampal pyramidal neurons) are not (Lancaster and Adams, 1986).

Ca^{2+} is a ubiquitous second messenger that interacts with a multitude of proteins and participates in the regulation of a vast number of signal transduction pathways.

Therefore, it is not surprising that cells make a concerted effort to tightly regulate their intracellular Ca^{2+} concentration and maintain a gradient that is higher than for any other ion. Ca^{2+}-activated K^+ channels have evolved to use this second messenger to regulate their opening and closing (gating) and to support the ability of the cell to finely regulate the amount of Ca^{2+} that is allowed to enter.

The activation of SK channels by Ca^{2+} resembles the binding of Ca^{2+} to Ca^{2+}-binding proteins, as they both have a fast Ca^{2+} response and a high Ca^{2+} sensitivity. However, SK-channel activation cannot be explained by Ca^{2+} binding directly to the channel, as there is no Ca^{2+}- binding motif in the primary structure of the SK subunits. Instead, the functional SK channels are heteromeric complexes with calmodulin, which is constitutively associated with the α-subunits in a calcium-independent manner (Xia et al., 1998). The role of calmodulin in SK-channel gating is generally accepted for all SK channels, and is supported by functional and biochemical evidence.

I.2.2. Pharmacology and physiology

Four genes comprise the SK channel family (SK1-SK4). This gene family can be divided into two subfamilies, SK1-3 and SK4. The genes that encode the SK channels 1-3 belong to the KCNN gene family. They were identified by database mining and subsequently isolated by homology cloning (Köhler et al., 1996). The SK1, 2 and 3 subunits are expressed in excitable tissues of the central nervous system. Their half-maximal activation occurs at cytosolic free Ca^{2+} concentrations in the range of 600-700 nM. Cloned SK channels show activation time constants of 5-15 ms with 10 μM Ca^{2+}. The present body of work will concentrate on the members of this first subfamily, namely

the SK2 subunit.

The second subfamily, the SK4 (also known as IK1) channel, differs from the other SK channels in that its transcript is only found in nonexcitable tissues such as placenta and lung. Its half-maximal activation takes place at 95 nM free Ca^{2+}. In view of this low concentration, it is likely that SK4 opens at resting levels of Ca^{2+} in certain types of cells. The hyperpolarization resulting from the activity of human SK4 suggests that this channel can regulate electrogenic transport (van Hahn et al., 2001).

SK channels have a similar topology to members of the voltage-gated (Kv) K^+ superfamily. They consist of six transmembrane segments (S1-S6), with the pore located between S5 and S6. The S4 segment in SK channels, which confers voltage sensitivity to the Kv channel, shows a reduced number and a disrupted array of positively charged amino acids (Köhler et al., 1996). These differences in the primary sequence could represent the molecular framework for the observed voltage-independence of SK channels.

Cloned SK channels are heteromeric complexes of SK channel subunits and calmodulin. SK channels are voltage-insensitive and their activation is dependent on increases in intracellular Ca^{2+} (IC_{50} = 300-700 nM). SK channels are not directly activated by Ca^{2+}. Their Ca^{2+} gating is conferred upon the constitutive binding of calmodulin to the carboxy-terminal region of each SK channel subunit (calmodulin-binding domain, CaMBD; Xia et al., 1998; Fanger et al., 1999 and Keen et al., 1999). Calmodulin is constitutively bound through its carboxy-terminal lobe to the proximal portion of the CaMBD (Keen et al., 1999). Upon Ca^{2+} binding to the calmodulin amino-terminal EF hands, a conformational rearrangement takes place in which the amino-

terminal lobe of calmodulin contacts the distal domain of the CaMBD on a neighboring subunit. It is suggested that this conformational change is transduced to the S6 transmembrane domains and subsequent rearrangements open the SK channel pore. According to this model, which is supported by structural data obtained by crystallization and NMR spectroscopy of the CaMBD bound to calmodulin, the SK channels may gate as dimers-of-dimers (Schumacher et al., 2001; Wissmann et al., 2002).

Pharmacological studies of SK channels have revealed that SK channels are specifically blocked by apamin, a peptide derived from the honey bee (*Apis melllifera)* venom (Blatz et al., 1986). However, there are differences in sensitivity among the channel subtypes, with SK2 channels being the most sensitive (IC_{50} < 0.1 nM), SK3 moderately sensitive (IC_{50} = 0.01-2 nM) and SK1 channels being the least sensitive (IC_{50} = 0.8-12 nM). SK channels are also blocked by the scorpion toxin tamapin from *Mesobuthus tamulus* (IC_{50} for SK2 channels = 24 pM), and the organic compounds d-tubocurarine (IC_{50} for SK2 channels = 2.4 μM) and bicuculline methiodide (IC_{50} for SK2 channels = 1.1 μM), and are activated by 1-ethyl-2-benzimidazoline (EBIO), which enhances the calcium sensitivity and open probability of SK channels (Pedarzani et al., 2001) and NS-309 (Strobaek et al., 2004). Except for Lei-Dab7, a drug, which preferentially blocks SK2 channels (Shakkotai et al., 2001), there are no pharmacological tools to differentiate between SK1, SK2 and SK3 channels.

Upon elevation of intracellular Ca^{2+}, SK channels activate and conduct an outward K^+ current. Consistent with this, apamin-sensitive outward K^+ currents have been observed in neurons in a variety of brain regions, including the cerebellum (Cingolani et al., 2002), lateral amygdala (Faber et al., 2002), subthalamic nucleus

(Hallworth et al., 2003) and hippocampus (Stocker et al., 1999). These apamin-sensitive currents participate in the afterhyperpolarization following action potential firing, as will be described later.

I.2.3. SK channel distribution in the central nervous system

Northern blot, in situ hybridization and RT-PCR analyses have revealed that SK1-3 are predominantly expressed in the nervous system (Köhler et al., 1996; Stocker and Pedarzani, 2000), whereas SK4 is mainly expressed in blood and epithelial cells (Ishii et al., 1997), and in some peripheral neurons (Böttger et al., 2002).

In situ hybridization, in accordance with immunohistochemical studies, has shown that in the mature rat central nervous system, SK1-3 channel subunits have partially overlapping, but clearly distinct distribution patterns, with SK1 and SK2 showing extensive colocalization, and SK3 relatively low levels of expression (Sailer et al., 2002; Stocker and Pedarzani, 2000). In particular, SK1 and SK2 are co-expressed at high levels in the neocortex (entorhinal cortex, subiculum, layer V neocortical pyramidal neurons), hippocampus (CA1-3 pyramidal neurons), thalamus (reticularis thalami and antedorsal neurons) and in some cranio motor neurons (motor trigeminal, facial, oculomotor and trochlear nuclei) (Stocker and Pedarzani, 2000; Sailer et al., 2004; Stocker et al., 1999). The subcellular localization of SK2 subunits differs in dissociated CA1 neurons, where the channels are mainly somatic (Bowden et al., 2001) and in fixed tissue, where they are absent from the soma and mainly expressed in the dendritic processes of CA1 neurons (Sailer et al., 2002, 2004). SK3 is maximally expressed in subcortical regions, including several thalamic nuclei (laterodorsal, mediodorsal, contralateral and central medial), the lateral septum, the medial habenula, the supraoptic

nucleus, several brainstem nuclei (dorsal vagal and ambiguous nuclei) and, interestingly, in many monoaminergic neurons (the dopaminergic substantia nigra pars compacta and ventral tegmental area, the serotonergic dorsal raphe nuleus and the noradrenergic locus coeruleus) (Stocker and Pedarzani, 2000; Sailer et al., 2002, 2004; Stocker et al., 1999). Interestingly, SK3 immunoreactivity was found predominantly in the terminal field of the mossy fibers and in fine varicose fibers, and in hippocampal cultures, SK3 was colocalized with presynaptic markers (Obermair et al., 2003). An overall similar distribution of SK channel subunits was also described in human (Rimini et al., 2000) and murine (Sailer et al., 2002) brain.

Stocker et al. (1999) have performed a detailed analysis of SK channel mRNA expression within the hippocampus. CA1 neurons express high levels of SK2, moderate levels of SK1 and low levels of SK2 mRNAs. Sailer et al. (2002) reported that SK2 immunoreactivity was highest in CA1/CA3 and particular apparent in the proximal dendrites. These findings are underscored by previous reports, which suggest that apamin-sensitive SK channels are located predominantly in proximal and distal dendrites of mammalian neurons (Cangiano et al., 2002). In addition, Ca^{2+}-activated K^+ channels have been reported in the dendrites of mammalian neurons (Andreasen et al., 1995, Sah et al., 1996). High levels of SK1 immunoreactivity were reported in dendritic processes throughout much of the hippocampus, including CA1; no immunoreactivity was detected in the pyramidal cell layer.

Apamin-sensitive currents are absent in CA1 neurons from transgenic mice that lack SK2 channels (Bond et al., 2004). This is consistent with previous suggestions based on the pharmacological profile of the current and the high specificity of apamin (Stocker

et al., 1999; Sailer et al., 2002). Therefore, it is likely that the SK channel modulation of hippocampal function is mediated specifically by SK2 channels.

Taken together, the results of these studies indicate that specific SK subunits contribute to neuronal excitability and function in different brain regions, and possibly, at the cellular level, in different neuronal compartments. These results suggest that dendrites and dendritic spines are an appropriate target for studying the role of SK2 in synaptic plasticity.

I.2.4. SK channel function in the central nervous system

Recent progress has been made in relating cloned SK channels to their native counterparts. These findings argue in favor of regulatory mechanisms conferring distinct and specific functional profiles to native SK channels with specific subunit compositions in different neurons.

There exist conflicting data on hippocampal SK single channel properties. One study of single SK channels in hippocampal pyramidal neurons reports a single channel conductance of 18-20 pS and a slightly lower sensitivity to Ca^{2+} (Lancaster et al., 1991). In a subsequent report it was indicated that hippocampal SK channels exhibit a typical conductance of 10 pS (in symmetrical potassium solutions) and that they are half-activated by ~0.6 μM Ca^{2+} (Hirschberg et al., 1999). These properties are very similar to cloned rat SK2 and human SK1 channels (Kohler et al., 1996; Hirschberg et al., 1998). In the presence of a fixed concentration of Ca^{2+}, hippocampal SK channels can spontaneously and rapidly switch between two open probabilities, a property observed with cloned rSK2 channels (Hirschberg et al., 1998).

In hippocampal pyramidal cells, SK channels are activated by a rise in intracellular Ca^{2+} such as occur during an action potential. Their opening leads to hyperpolarization, which in turn decreases the action potential firing rate. Since neurons use action potential frequency to encode information, changes in the activity of SK channels should strongly affect signal processing.

The SK channel toxins apamin and scyllatoxin both suppress selectively the medium current I_{AHP}, without affecting the slow I_{AHP} in CA1 neurons. Furthermore, in current clamp experiments blockade of SK channel function produces a small increase in the number and initial frequency of action potentials elicited both by short (400 ms) and long (800 ms) current pulses of different amplitudes, but has no effect on spike frequency adaptation (Stocker et al., 1999; Stackman et al., 2002).

Several groups demonstrated that blockade of SK channels by apamin facilitates the induction of LTP in the CA1 area of rats (Behnisch et al., 1998; Norris et al., 1998) and mice (Stackman et al., 2002). Given that blockade of SK channels increases neuronal excitability, this facilitation of synaptic plasticity is most likely mediated by lowering the threshold for the induction of hippocampal synaptic plasticity.

Specific roles of distinct SK channel subtypes are starting to merge in different neuronal subtypes: there is no evidence for SK3 subunits being involved in regulating excitability and firing of hippocampal pyramidal neurons, where instead SK2 subunits have been associated to play a role, but interestingly, immunohistochemistry showed that SK3 is localized in presynaptic terminals at the neuromuscular junction (Roncarati et al., 2001) and in hippocampal pyramidal cultures (Obermair, 2003). The predominant SK subunit expressed in hippocampus is SK2 (Stocker et al., 1999, Stocker et al., 2000);

these findings support the hypothesis that SK2 subunits participate in the formation of the channels underlying the apamin-sensitive I_{AHP} in hippocampal pyramidal neurons. More recently, Cai et al. (2004) have shown that dendritic plateau potentials were slowed in their repolarization and prolonged in their duration by manipulations that inhibit SK2 channels. Focal application of the specific SK channel blocker apamin revealed that the channels responsible for this effect are expressed locally in the distal dendrites themselves. Lastly, the possible contribution to cellular function of SK1, expressed both in hippocampal pyramidal neurons and dentate gyrus granule cells, still remains unclear.

I.2.5. Distinct Ca^{2+} microdomains and SK2 populations in dendritic spines of CA1 neurons

There has been substantial recent progress in understanding dendritic processing in cortical and hippocampal pyramidal cells. The architecture of dendritic spines is unique. Dendritic spines protrude from the surface of many neurons and serve as the main targets for excitatory synaptic input (Malenka and Nicoll, 1999), thus mediating the transfer of synaptic potentials to the dendritic shaft and then to the soma for integration. More than 90% of excitatory synapses terminate on spines, with each spine bearing one synapse (Nimchinsky et al., 2002); the human brain therefore contains $>10^{13}$ spines. Dendritic spines are specialized structures, i.e. diffusionally isolated compartments (Svoboda et al., 1996) with low endogenous Ca^{2+} buffer capacity that allows large and extremely rapid changes in Ca^{2+} concentration (Sabatini et al., 2002). They are compact compartments that are connected to the dendritic shaft by a very thin neck structure that quite effectively limits Ca^{2+} diffusion, providing a discrete Ca^{2+} signaling zone (Svoboda

et al., 1996, Sabatini et al., 2002). Ca^{2+} enters dendritic spines through two pathways: NMDA receptors and voltage-dependent Ca^{2+}-channels. Imaging techniques have shown that in CA1 neurons, relatively weak synaptic stimulation leads to Ca^{2+} influx in spines primarily through NMDA receptors, while more physiological protocols that pair subthreshold stimuli with timed action potentials additionally activate voltage-dependent Ca^{2+}-channels (Köster et al., 1998, Yuste et al., 1999). Under some conditions, Ca^{2+}-induced Ca^{2+} release may also play a role (Emptage et al., 1999). However, NMDA receptors clearly play a major role in spine Ca^{2+} dynamics, as several groups have shown that blocking NMDA receptors abolishes spine Ca^{2+} transients, and that blockade of voltage-dependent Ca^{2+} channels does not reduce subthreshold synaptic Ca^{2+} transients in acute brain slices (Kovalchuk et al., 2001). In addition, because of the long time course of NMDA receptor-mediated Ca^{2+}-currents compared with those produced by action potentials, the NMDA receptor-mediated Ca^{2+}-transient will, compared with action potential-evoked one, appear relatively larger as more Ca^{2+} indicator is introduced (Sabatini et al., 2002). There is growing evidence that these Ca^{2+} sources are sequestered in highly organized, discrete Ca^{2+} signaling microdomains.

Recent models for synaptic integration and plasticity suggest that the physiologically relevant mechanisms require NMDA receptor activity evoked by high frequency synaptic stimulation alone, with temporally coordinate excitatory postsynaptic potentials (EPSPs) or with back-propagating action potentials. The distal dendrites of CA1 neurons undergo regenerative action potential-like events, termed plateau potentials, which are confined to the local dendritic branch in which they are triggered, enabling each compartment to act as a semi-autonomous synaptic integrator. Voltage-dependent

Ca^{2+}-channels account for most of the Ca^{2+} influx that underlie these plateau potential events. Focal glutamate application combined with dendritic imaging and whole cell recordings from CA1 neurons in the cultured slice preparation show that SK channels activated by Ca^{2+} influx through voltage-dependent Ca^{2+} channels exert a re-polarizing influence that shortens the plateau potential and deactivates the voltage-dependent Ca^{2+} channels (Cai et al., 2004). Thus, SK channels expressed locally in the distal dendrites contribute to the repolarization of Ca^{2+}-mediated plateau potentials of these neurons, indicating a prominent role in dendritic integration.

I.2.6. Blocking SK channels with apamin in intact animals: effects on behavior, learning and memory

Apamin, a toxin derived from honey-bee venom, is an octadecapeptide with two disulphide bonds (Bystrov et al., 1980). Apamin is a highly potent and selective blocker of SK channels, although there are differences in sensitivity among the different channel subtypes (based on radioligand binding assays: SK1: ~390 pM; SK2: ~3 pM; SK3: ~11 pM; Grunnet et al., 2001; based on functional assays: SK1: ~3.3–8.0 nM; SK2: ~0.06–0.14 nM; SK3: ~1.0–2.0 nM; Shah and Haylett, 2000; Strobaek et al., 2000). Importantly, SK channels are the only known apamin receptors, and therefore, the consequences of apamin application to cells heterologously expressing cloned SK channels, brain slices, and behaving animals may be attributed to SK channel blockade. The suggestion that apamin may affect cognitive function came from studies using radioiodinated apamin that showed that systemically administered apamin crosses the blood–brain barrier (Habermann, 1984), and that there are high densities of apamin- binding sites (SK

channels) in several learning and memory-related brain areas, such as the septum, the hippocampal formation, cingulate cortex and anteroventral thalamic nuclei (Mourre et al., 1987; Gehlert and Gackenheimer, 1993). These binding studies are further supported by in situ hybridization and immunohistochemical analyses that showed a coincidence between apamin- binding studies and the expression of the SK channel mRNAs and proteins (Stocker et al., 1999; Stocker and Pedarzani, 2000; Sailer et al., 2002; Sailer et al., 2004). Moreover, apamin-binding sites are affected by learning disorder diseases in humans. Ikeda et al. (1991) found that in patients with Alzheimer's disease there was a marked and highly selective loss of apamin binding sites from the subiculum and CA1 areas of the hippocampus. The smaller maximal binding value without alteration in K_d in CA1 neurons of one patient suggests that the reduction of apamin binding reflects a change in the number of apamin binding sites rather than a change in the binding properties of apamin. Significant reductions in apamin binding in Alzheimer's patients were restricted to the two hippocampal regions (subiculum and CA1) in which the levels of apamin binding were greatest, whereas apamin binding in other hippocampal regions in Alzheimer's patients was not different from that in controls. In areas such as CA3, neither the maximum binding value nor K_d appeared to be altered in patients with Alzheimer's disease.

The physiological consequences of systemic apamin administration have also been investigated and there are several inconsistencies in the literature concerning the effects of apamin on cognitive function in rodents. Several studies have reported that apamin is effective in improving the memory performance of mice and rats. Apamin was shown to facilitate memory processes in an appetitive-motivated bar-pressing response in

mice (Messier et al., 1991). Apamin administration (0.2 mg/kg i.p.) either before or after the training accelerated the acquisition of the bar-pressing response. In another study (Deschaux et al., 1997), apamin administration (0.4 mg/kg i.p.) improved learning in an object recognition task in rats only when it was injected before acquisition but not when injection took place just after the acquisition session or before the restitution session. Apamin administration (0.06 mg/kg i.p. before the training) has also been shown to reverse the spatial navigation defect during the initial and reversal learning stages of the water maze task induced by a medial septal lesion (Ikonen et al., 1998). Finally, a recent study presented evidence that apamin improves consolidation in an olfactory associative learning task involving new odor-reward association (Fournier et al., 2001). However, apamin does not seem to have any effects on passive avoidance behavior (Deschaux et al., 1997; Gheraldini et al., 1998), delayed matching to position task (Poorheidari et al., 1998) or Morris water maze performance when measured after extensive training (Ikonen et al., 1998; van der Staay et al., 1999) of intact mice or rats. Since apamin is effective in some paradigms but not in others, it appears that apamin-sensitive channels affect only certain circuitries involved in memory processing. Differential effects of apamin on distinct stages of memory may explain the discrepancies between these sets of data. Alternatively, it has been suggested (Stackman et al., 2002; see below for a detailed discussion of that paper), that apamin exerts its influence at an early stage of memory encoding, an effect that may not have been detected given many of the approaches used previously. This indicates that apamin facilitated memory after minimal spatial or nonspatial training. Apamin did not have a significant effect on memory retention in mice after extensive spatial training, consistent with the above reports of the effects of apamin

in mice and rats (Ikonen et al., 1998; van der Staay et al., 1999).

Heurteaux et al. described one mechanism that might contribute to the effects of apamin administration. They reported that apamin administration increased the expression of the immediate early genes c-fos and c-jun in the hippocampus (Heurteaux et al., 1993). These genes are thought to be involved in the initial activation of neurons during the memory process and thus are markers for memory formation (Morgan and Curran, 1989), suggesting that the apamin-induced increase of immediate early gene expression might be related to the apamin-induced facilitation of learning.

As discussed in detail below (see section I.3.) SK channels contribute to the generation of the afterhyperpolarization (AHP; for a detailed discussion see below), and a learning-induced reduction of the AHP has been shown to correlate with learning and memory in other behavioral paradigms. A potentiation of excitatory postsynaptic potentials (EPSPs) and a reduction in the medium AHP and slow AHP currents is associated with classical conditioning of the eye-blink response in rabbits (Moyer et al., 1996; Disterhoft et al., 1988; Coulter et al., 1989) and with olfactory operant conditioning in rats (Saar et al., 1998). For example, in the study of Moyer and others, CA1 neurons recorded from conditioned rabbits exhibited a learning-induced decrease in post-burst afterhyperpolarizations and reduced spike-frequency adaptation. These increases in neuronal excitability were specific to learning since they were not observed in pseudo-conditioned animals or "poor learners". Similar reductions in hippocampal CA1 post-burst AHPs have also been observed in rats trained in an olfactory discrimination task, in which they had to memorize odors in order to be rewarded with drinking water (Saar et al., 2001; Saar et al., 2003; Zelcer et al., 2005). These training paradigms result in rule

learning, which enables enhanced acquisition of odor memory and also resulted in increased neuronal excitability, as indicated by reduced afterhyperpolarization. In addition, the recent study of Zelcer et al. (2005) shows that rats that learned an olfactory discrimination task subsequently exhibited improved learning in a novel hippocampal-dependent task – the Morris water maze, suggesting that alterations of intrinsic excitability in CA1 neurons resulted from olfactory discrimination learning and effectively altered processes underlying hippocampal-dependent learning. Morris water maze learning is a paradigm designed to target hippocampal-dependent spatial learning, and is sensitive to deficits accompanying lesions of the hippocampus (Morris et al., 1982). During this task, the animals are placed in a circular tank filled with opaque water, and are required to swim to a hidden platform in order to escape. Successful acquisition of this task requires the animals to remember the placement of the platform location by using extramaze cues to escape the water quickly and effectively. Acquisition of the Morris water maze requires the hippocampus to form spatial associations between the platform location and the surrounding environment, making the hippocampus necessary for this task (Wallenstein et al., 1998). It was concluded that enhancement of neuronal excitability and reduction in post-burst AHP accompany acquisition of enhanced learning capability. These long-term changes in intrinsic neuronal properties occur throughout the neuronal populations that are relevant for the learned tasks (piriform cortex for olfactory learning and hippocampus for the classical conditioning of the eyeblink response). The AHP amplitude in neurons from hippocampus and piriform cortex tends to return to its initial value within days when training is suspended. This recovery is not accompanied by memory loss, but strongly affects the newly acquired enhanced learning capability.

Together, these data suggest that enhanced neuronal excitability underlies rule learning, but is not the biophysical trace of long-term memory for specific sensory inputs.

Recent behavioral studies from our group were designed to explicitly test the influence of apamin on the initial stage of spatial memory formation or encoding (Stackman et al., 2002). Naive mice received apamin (0.4 mg/kg, i.p.) or 0.9% saline 30 minutes before daily training for 6 days (four trials per day) in the water maze task. Apamin-treated mice exhibited faster learning of the platform location during the initial training trials in the Morris water maze. Probe tests in which the platform was removed from the pool were used to test spatial search behavior at early, intermediate, and late stages of memory encoding. Apamin-treated mice exhibited significant spatial memory after minimal training, whereas control mice exhibited chance levels of performance. There were no further differences in performance on subsequent probe tests after more extensive training. These data suggest that blocking SK channels facilitated the encoding of spatial memory and differ from previous water maze studies in which spatial memory was not probed until after extensive training (Ikonen et al., 1998; Ikonen and Riekkinen 1999; van der Staay et al., 1999). The effects of apamin on spatial memory encoding may be specific to the hippocampus because apamin did not influence memory encoding in a hippocampal-independent version of the water maze task. Together, these data suggest that blockade of SK channels facilitates an early stage of hippocampal-dependent spatial memory. This interpretation is supported by the recent study of Hammond et al. (2006), in which overexpression of SK2, the subtype expressed in CA1 neurons, drastically impaired learning in two hippocampal-dependent tasks. SK2 overexpression disrupted hippocampal-dependent learning and memory in both the Morris water maze and a

contextual fear conditioning paradigm. In the Morris water maze, SK2 overexpressing mice exhibited profound learning impairments, and were unable to learn and retain information about the platform location even when the training program included a visible platform phase and was markedly extended to 11 days of training. In addition, SK2 overexpression disrupted both hippocampal-dependent contextual fear conditioning and hippocampal-independent cued fear conditioning. The impairment of SK2 overexpressing mice in cued fear conditioning, an amygdala-dependent task, indicated that SK2 channels might also influence emotional memory formation. SK2 channel modulation of amygdala function is an interesting possibility, since SK2 channels are expressed throughout this region (Sailer et al, 2002; Sailer et al., 2004) and since it has been shown that – similar to hippocampal synaptic plasticity and learning – SK channels in the lateral amygdala regulate the activation of NMDA receptors (Faber et al., 2005).

The prevailing model for the cellular basis of learning and memory is that experience-driven changes in synaptic activity result in long-term changes in synaptic strength (synaptic plasticity). Several studies have investigated the contribution of SK channels to long-term synaptic plasticity. An initial study used apamin to block SK channels in brain slices and measured field potentials in area CA1 of the hippocampus in response to high frequency stimulation (a single 100 Hz tetanus) of the Schäffer collateral axons. The results showed that the resulting increase in the field EPSP was intensified by extracellular application of apamin (Behnisch and Reymann, 1998). It was proposed, that the apamin-mediated facilitation of LTP was caused at least in part, by changes in the excitability of interneurons, which at this time, were the only cells on the CA1 region known to express an apamin-sensitive AHP. Taken together, SK channel blockade

facilitated the induction of long-term potentiation (LTP) in the hippocampal formation and this would correlate behaviorally with accelerated hippocampus-dependent spatial and non-spatial memory encoding (Stackmann et al., 2002). In a study of age-related alterations in synaptic plasticity, blockade of L-type Ca^{2+} channels by nifedipine facilitated the induction of synaptic plasticity in Schäffer collateral CA1 synapses in slices from aged rats (Norris et al., 1998; Foster et al., 1999). Using field recordings of area CA1 of the hippocampus, the authors explored the influence of L-type Ca^{2+} channels on the induction of CA3/CA1 synaptic plasticity during aging, and demonstrated that, in aged rat slices, the L-type Ca^{2+} channel antagonist nifedipine reverses the susceptibility to induction of LTD using 1 Hz stimulation and enhances the induction of NMDA receptor-dependent synaptic enhancement using 5 Hz stimulation. This is an example in which low frequency stimulation, which normally causes LTD, at least in adult animals caused potentiation in nifedipine-treated aged animals. It was concluded that the facilitation of synaptic plasticity was mediated by a reduction in SK channel activity because application of apamin mimicked the effects of nifedipine. The authors suggested that an age-related increase in SK channel activity might underlie impaired plasticity and memory in aging.

Two studies from our group shaped the overriding hypothesis that I tested in my thesis work. First, Stackman and colleagues (2002) combined field recordings of area CA1 of the hippocampal brain slices with behavioral tasks, as discussed above, to test the hypothesis that apamin block of SK channels facilitated the induction of synaptic plasticity and hippocampal-dependent learning. In this study, apamin was applied to mouse hippocampal slices and synaptic plasticity was examined after a variety of

conditioning frequencies (1-100 Hz). The effect of apamin was to shift the modification threshold to lower frequencies, facilitating the induction of synaptic plasticity. Stimulation frequencies that do not induce synaptic plasticity in control slices (i.e., 5 Hz and 25 Hz) produce LTD and LTP, respectively, in the presence of apamin. The mechanism was postsynaptic and required NMDA receptor activation. These studies were performed in the presence of picrotoxin, eliminating a role for an effect via GABAergic interneurons. In concert with the brain slice studies, systemic apamin administration facilitated learning in the Morris water maze and the spontaneous recognition task, respectively, but did not alter memory retention. Indeed, the effects of systemic apamin injection reflected the shift of the modification threshold in brain slice experiments; the control and apamin-treated mice learned to the same finale extent, but apamin promoted earlier acquisition, e.g. shifted the response to lower trials. The parallel between the reduction of the threshold for synaptic plasticity and the improved memory encoding after minimal spatial or non-spatial training suggests a correlation between the facilitation of the induction of synaptic plasticity and learning. In line with the findings of this study, Hammond et al. (2006) recently found that SK2 overexpression impairs the induction of LTP in a frequency-dependent manner via a postsynaptic mechanism without affecting the induction of LTD. Specifically, SK2 overexpression reduced LTP elicited with 50 Hz high frequency stimulation. However, SK2 overexpression did not disrupt LTP elicited with a 100 Hz, high frequency stimulation, nor did it disrupt LTD elicited with a 1 Hz low-frequency stimulation. Therefore, the specific effect of SK2 channel overexpression on plasticity was observed after an intermediate frequency stimulus (50 Hz). This suggests that SK2 channel regulation of synaptic plasticity may

serve as a mechanism to maintain the direction of synaptic plasticity. Taken together, studies with apamin have shown that SK2 channel block enhances learning and memory in hippocampal-dependent tasks (Stackman et al., 2002), while increased SK channel activity (via SK2 overexpression) impairs learning and memory in hippocampal-dependent tasks (Hammond et al., 2006).

Most importantly, the study by Stackman and others gave rise to a specific hypothesis by which SK channels regulate hippocampal synaptic plasticity and learning and memory. During baseline synaptic transmission the excitatory neurotransmitter glutamate is released from the presynaptic bouton and activates two primary ionotropic coupled receptors, the AMPA and NMDA receptors. The AMPA receptors are generally seen as providing the primary depolarization associated with synaptic activation, while the NMDA receptors are viewed as a secondary source of depolarization, being primarily involved in plasticity. During normal synaptic transmission, Na^+ flows only through AMPA receptors, because extracellular Mg^{2+} blocks the conduction of NMDA receptors. Depolarization of the postsynaptic membrane expels Mg^{2+} from the NMDA receptor channel, allowing it to conduct a mixture of Na^+, K^+ and Ca^{2+} ions. The resulting increase in Ca^{2+} within the dendritic spine is the critical trigger for synaptic plasticity. Based on the previous results from our group, we propose that the increases in intracellular Ca^{2+} through NMDA receptors activate SK channels. SK channel activation repolarizes the postsynaptic neuron and suppresses NMDA receptor activity and Ca^{2+} influx through NMDA receptors by re-imposing Mg^{2+} blockade, thereby tuning changes in synaptic strength. (Postsynaptic depolarization induced by repetitive synaptic stimulation increases intracellular Ca^{2+} and activates SK channels. By hyperpolarizing the

postsynaptic membrane, SK channels decrease excitability and may modulate the activation of NMDA receptors, which involves voltage-dependent removal of the Mg^{2+} block (Mayer et al., 1988). By affecting the degree of NMDA receptor activation and the subsequent Ca^{2+} entry, SK channels modulate the induction of synaptic plasticity.) This model can account for recently reported effects of apamin on hippocampal plasticity and memory. However, apamin also facilitates several forms of non-hippocampal memory (Messier et al., 1991; van der Staay et al., 1999), suggesting that SK channels can exert similar effects in non-hippocampal memory circuits that employ NMDA receptor-dependent plasticity.

I.3. The afterhyperpolarization (AHP)

I.3.1. Overview

A prominent feature in many excitable cells under current-clamp conditions is a substantial afterhyperpolarization that regulates the excitability of the cell for periods ranging from a few milliseconds up to several seconds. A burst of action potentials is usually succeeded by a prolonged afterhyperpolarization that is functionally inhibitory and associated with a conductance increase. Afterhyperpolarizations that follow a single spike or a spike train can mediate different forms of feedback regulation of excitability. Potassium channels are particularly important for negative feedback regulation. Once activated by the action potential – i.e. by the ensuing depolarization, Ca^{2+} influx, or both – K^+ channels mediate outward currents that outlast the action potentials and tend to hyperpolarize the cell, thus generating afterhyperpolarizations. These AHP-generating K^+

currents are essential determinants of refractoriness, interspike duration and trajectories and, hence, spike timing, discharge patterns and discharge frequencies.

Neurons in the vertebrate central system are equipped with a variety of AHP-generating K^+-currents and afterhyperpolarizations. Depending on the time course of the currents, they can contribute to the repolarization of the action potential, or to one or more phases of the afterhyperpolarization (fast, medium and slow AHP) following single or bursts of action potentials. The medium and slow AHPs play a major role in the generation of spike frequency adaptation, a feature of many neurons. Spike frequency adaptation occurs when a steady stimulus induces repetitive firing that gradually changes from high to low frequency or eventually stops in spite of the persistent input (Hille, 2001). Spike frequency adaptation is an important factor in synchronizing neuronal populations and setting the frequency of population rhythms (Crook et al., 1998, Fuhrman et al., 2002).

The different kinetic and pharmacological properties of Ca^{2+}-activated K^+-currents present in different or sometimes co-existing in the same neurons reflect the existence of different types of Ca^{2+}-activated K^+-channels. Although this afterhyperpolarization pattern was first described almost two decades ago, important issues remain partly unresolved or controversial, in particular regarding the mAHP mechanism. In particular it is widespread perception, that the hippocampal mAHP current is mediated largely by SK-type Ca^{2+}-activated K^+ channels (Sah, 1996; Bond et al., 1999). For example, two recent studies support this notion (Stocker et al., 1999; Stackman et al., 2002), and apparently contradict previous and recent studies that found no SK channel contribution to the mAHP (Storm, 1989; Gu et al., 2005).

The goal of the second aim of this thesis was to re-examine the mAHP mechanism.

I.3.2. The fast afterhyperpolarization

The current underlying the fAHP has been named I_C. This current is voltage dependent (Adams et al., 1982) and is blocked by low concentrations of TEA, iberiotoxin, and paxilline indicating that the underlying channels are BK-type channels (Adams et al., 1982; Lancaster and Nicoll, 1987; Shao et al., 1999).

BK channels were the first type of Ca^{2+}-activated potassium channel to be cloned from *Drosophila*, as the product of the *slowpoke* gene (Adelman et al., 1992; Atkinson et al., 1992). Subsequent studies led to the characterization of similar currents in vertebrate peripheral and central neurons, where I_C contributed to the repolarization of action potentials and mediated the fast phase of the afterhyperpolarization (Adams et al., 1982, Lancaster and Nicoll, 1987, Storm, 1987).

BK channels have a single channel conductance in the range of 100 pS under physiological conditions, which can rise up to 250 pS when the extracellular potassium concentration equals the intracellular concentration. They are activated by voltage and Ca^{2+} modulates their open probability. At membrane potentials near the resting potential (-50 mV to -70 mV), these channels require 1-10 μM Ca^{2+} for activation (Sah, 1996). BK channels carry out at least two important functions in neurons. In the soma, they are thought to participate in action potential repolarization and generate the fAHP (Lancaster et al., 1987), thus contributing to set the rate of action potential firing. In presynaptic terminals, they regulate the duration of the action potential and limit Ca^{2+} entry, thereby

modulating neurotransmitter release.

Additionally, it has been observed that the fast AHP following an action potential, while present in the soma, was absent in the dendrites (Poolos et al., 1999). Furthermore, as had been shown previously, the duration of the dendritic action potential was larger in the soma and the rate of repolarization was considerably slower. Therefore, the conclusion drawn from these experiments is that the action potential becomes progressively wider and slower to repolarize as it back-propagates into the dendrites, at least in part because of a lack of BK channels in distal dendrites.

I.3.3. The medium afterhyperpolarization

Following the action potential and the fast AHP, many neurons display a prolonged afterhyperpolarization lasting between hundreds of milliseconds and seconds. Depending on the neuronal subtypes, this AHP can be dissected into two main components, a medium and a slow AHP. The medium AHP (mAHP) follows single or bursts of action potentials, and presents a relatively fast activation ($\leq$5 ms) and a time constant of decay in the range of hundreds of milliseconds (Gustafsson and Wigstrom, 1981; Schwindt et al., 1988 and Storm, 1989). I_{mAHP} decays with a time course dependent on the amount of Ca^{2+} influx; as Ca^{2+} load increases, peak amplitude increases and the current decay is slowed (Sah, 1992).

Depending on the neuronal subtype, up to three different K^+ currents contribute to the generation of the mAHP: the voltage-dependent I_M (CA1 pyramidal neurons, Storm, 1989), the Ca^{2+}- and voltage-dependent I_C (CA1 pyramidal neurons, Lancaster and Nicoll, 1987 and Storm, 1989), and the purely Ca^{2+}-activated I_{AHP} blocked by the bee

venom toxin apamin (bullfrog and rat sympathetic neurons, Pennefather et al., 1985 and Kawai and Watanabe, 1986; neocortical pyramidal neurons, Schwindt 1988; locus coeruleus neurons, Osmanovic et al., 1990). The medium I_{AHP} is present in a large number of neurons, in agreement with the widespread distribution of apamin binding sites in rat brain sections (Mourre et al., 1986 and Gehlert and Gackenheimer, 1993; Sah, 1996). Depending on the neuronal subtypes and their contingent of ion channels, the function of the medium I_{AHP} varies from contributing to the instantaneous firing rate as in layer V sensorimotor cortical neurons (Schwindt et al., 1988), to setting the tonic firing frequency as in dorsal vagal neurons (Sah and McLachlan, 1992), to regulating burst firing and rhythmic oscillatory activity in midbrain dopaminergic neurons (Seutin et al., 1993), in nucleus reticularis thalami neurons (Bal and McCormick, 1993), in inferior olive neurons (Bal and McCormick, 1997), and in hypothalamic supraoptic neurons (Hu and Bourque, 1992). Neonatal cerebellar Purkinje neurons express an apamin-sensitive IAHP important for the intrinsic firing pattern of these cells and mediated by homomeric SK2 channels, as deduced from in situ hybridization, immunohistochemistry and pharmacological evidence (Cingolani et al., 2002). In the subthalamic nucleus, SK channels have recently been shown to play a pivotal role in regulating the spontaneous and network-driven firing activity of the neurons (Hallworth et al., 2003).

The medium AHP has pharmacological properties similar to recombinant SK2 channels, consistent with the hypothesis that SK2 channels generate this AHP component. SK2 channels specifically underlie the apamin-sensitive I_{mAHP} in the hippocampus, since the ImAHP is absent in CA1 neurons of transgenic knockout mice lacking SK2 channels (Bond et al., 2004). Given their biophysical (voltage-insensitivity,

Ca^{2+} sensitivity) and pharmacological (sensitivity to apamin) features, SK channels are ideal candidates to underlie the mAHP in the central and peripheral nervous system. Consistent with their contribution to the mAHP, in hippocampal CA1 neurons, blockade of SK channels with apamin enhances cell excitability (Stocker et al., 1999).

Recently, Gu et al. (2005) aimed at re-examining the mechanisms underlying the mAHP, the authors conclude that M- and h-channels generate the somatic mAHP in hippocampal pyramidal cells, with little or no net contribution from SK channels. It is difficult to reconcile these results with the studies mentioned above.

In summary, the contribution of SK channels to the generation of the mAHP in CA1 pyramidal neurons has been observed in some studies, but not in others.

I.3.4. The slow afterhyperpolarization

The current underlying the slow AHP is generated by Ca^{2+}-activated K^+-channels of unknown molecular composition (Vogalis et al., 2003). The current that underlies the slow AHP was first described in neurons in the myenteric plexus (Hirst et al., 1985). Following Ca^{2+} influx, this current has a time to peak on the order of hundreds of milliseconds, and decays to baseline with a time constant of 1–2 s at 30°C. As with I_{mAHP}, this current requires a rise in cytosolic Ca^{2+} for activation and is voltage insensitive. I_{sAHP} is not blocked by apamin or low concentrations of TEA. However, I_{sAHP} is modulated by a range of neurotransmitters including noradrenaline, serotonin, glutamate, and acetylcholine all of which block the current (Nicoll, 1988).

What we currently know is that I_{sAHP} is mediated by voltage-independent Ca^{2+}-activated K^+ channels of small conductance, 2–7 pS in physiological K^+ gradient, as

inferred from noise analysis studies in hippocampal pyramidal neurons and dentate gyrus granule cells (Sah and Isaacson, 1995; Valiante et al., 1997). A current resembling the I_{sAHP} present in hippocampal and cortical neurons has also been characterized in myenteric neurons, where it is modulated by the cAMP-protein kinase A pathway as in the brain (Vogalis et al., 2003). However, the single channels mediating I_{sAHP} in myenteric neurons have a higher conductance, 10–17 pS in physiological K^+ gradient (Vogalis et al., 2001) and 60 pS in symmetric K^+ (Vogalis et al., 2002), compared to the one estimated for sAHP channels in central neurons. At this stage it is therefore not clear whether the sAHP currents measured in cortical and myenteric neurons are mediated by the same channels.

A second relevant point is the activation of I_{sAHP} by Ca^{2+}. Although already early studies showed the dependence of I_{sAHP} on Ca^{2+} (Hotson and Prince, 1980; Schwartzkroin and Stafstrom, 1980; Gustafsson and Wigstrom, 1981; Madison and Nicoll, 1984; Lancaster and Adams, 1986), I_{sAHP} presents a slow time course of activation and reaches its peak amplitude up to 0.5 s following the end of Ca^{2+} entry (Lancaster and Adams, 1986). In response to the activation of voltage-gated Ca^{2+} channels, intracellular free Ca^{2+} rises much faster both in the neuronal soma and in the dendrites (Jaffe et al., 1992; Markram et al., 1995; Lasser-Ross et al., 1997; Sah and Clements, 1999), thereby revealing a mismatch between the kinetics of intracellular Ca^{2+} concentration and those of I_{sAHP}: Suggested reasons for the slow kinetics of I_{sAHP} include slow diffusion of Ca^{2+} to a site distant from its point of origin (Lancaster and Nicoll, 1987; Lancaster and Zucker, 1994; Zhang et al., 1995), Ca^{2+}-induced Ca^{2+}-release (Sah and McLachlan, 1991; Moore et al., 1998; Tanabe et al., 1998; but see Zhang et al., 1995; Lasser-Ross et al., 1997), a

possible involvement of an enzymatic step in the activation of sAHP channels (Schwindt et al., 1992; Lasser-Ross et al., 1997; Moore et al., 1998), delayed facilitation of the Ca^{2+} channels supplying the Ca^{2+} for the activation of I_{sAHP} (Bowden et al., 2001) or intrinsically slow activation and deactivation kinetics of the channels underlying I_{sAHP} (Schwindt et al., 1992; Sah, 1993; Sah and Clements, 1999). Experimental evidence and arguments have been produced in the years in support or against each of these theories, but the issue of the dissociation between the kinetics of neuronal Ca^{2+} transients and those of I_{sAHP} is still open.

Functionally the I_{sAHP} is responsible for the late phase of spike frequency adaptation and leads to a strong reduction or a complete cessation of action potential firing, thereby controlling the repetitive firing of neurons and limiting the numbers of action potentials generated in response to stimuli. A trademark feature of the I_{sAHP} that has been studied in different peripheral and central neurons is its modulation by several neurotransmitters and second messenger pathways. When I_{sAHP} is suppressed as a consequence of neurotransmitter-induced activation of PKA, the neurons are more excitable and will follow incoming signals more faithfully. This neuromodulatory effect can be regarded as a molecular correlate of paying attention.

SPECIFIC AIMS

Aim 1: Determine whether Ca^{2+} influx via NMDA receptors activate SK2 channels, thereby modulating the shape of the EPSP. The synaptically evoked Ca^{2+} entry might be expected to activate dendritic SK channels located near the synapse. If so, the Ca^{2+} increase, which outlasts the postsynaptic potential by several hundreds of milliseconds, should lead to a long-lasting SK channel conductance increase. This in turn would be expected to locally shunt subsequent dendritic EPSPs, which would thus contribute less to the depolarization of the cell soma and initial segment than the first EPSP. Our working hypothesis was that Ca^{2+} influx through dendritic spine NMDA receptors activates SK2 channels in CA1 neurons, forming a local regulatory circuit. To test this hypothesis, experiments in the whole-cell current-clamp configuration of CA1 neurons in brain slices from wild type mice were performed, in which we attempted to evaluate the effect of the SK channel blocker apamin on evoked subthreshold EPSPs, to determine whether activation of SK2 channels by Ca^{2+} influx via NMDA receptors modulates the shape of the EPSP, and furthermore to study whether SK2 exerts its effect via a pre- or a postsynaptic mechanism.

Aim 2: Determine whether SK2 channels contribute to the afterhyperpolarization in hippocampal pyramidal CA1 neurons. The goal of this study was to characterize the origin of the afterhyperpolarization associated with single or bursts of action potentials: in the whole-cell current-clamp configuration of CA1 neurons in brain slices from wild type mice, we sought to determine the effect of the SK channel blocker apamin on the

medium component of the afterhyperpolarization and on the interspike afterhyperpolarization.

SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines

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ABSTRACT

Small conductance Ca^{2+}-activated K^+ channels (SK channels) modulate excitability and influence the induction of synaptic plasticity at hippocampal CA3 to CA1 synapses. We find that SK channels are localized to dendritic spines and their activity reduces the amplitude of evoked synaptic potentials in an NMDA receptor (NMDAr) dependent manner. Using combined 2-photon-laser scanning microscopy and 2-photon laser uncaging of glutamate, we show that SK channels regulate NMDAr-dependent Ca^{2+} influx within individual spines that contain active synapses. SK channels are tightly coupled to synaptically activated Ca^{2+} sources and their activity reduces the amplitude of NMDAr dependent Ca^{2+} transient. These effects are mediated by a feedback loop within the spine head such that during an EPSP, Ca^{2+}-influx opens SK channels that repolarize the membrane and promote rapid Mg^{2+} block of the NMDAr. Thus blocking SK channels facilitates the induction of long-term potentiation by enhancing NMDAr-dependent Ca^{2+} signals within dendritic spines.

INTRODUCTION

In CA1 hippocampal neurons, apamin-sensitive small conductance Ca^{2+}-activated K^+ channels (SK channels) modulate firing frequency by contributing to the afterhyperpolarization (AHP) that follows an action potential[1-3]. Additionally, blocking SK channels with apamin facilitates the induction of long-term potentiation (LTP) at Schäffer collateral to CA1 synapses. Apamin shifts the point of inflection between long-term depression (LTD) and long-term potentiation (LTP), such that lower stimulus frequencies[4] can generate LTP. Results from behavioral experiments parallel the effects induced by apamin in brain slices. Apamin crosses the blood-brain barrier[5] and intraperitoneal injection facilitates the acquisition of hippocampal-dependent learning tasks such as locating the hidden platform in the Morris water maze, and novel object recognition[4].

Several forms of long-term plasticity at the CA3 to CA1 synapses are triggered by Ca^{2+} influx through NMDAr and the kinetics and amplitude of evoked Ca^{2+} transients in the postsynaptic cell determine the direction and extent of synaptic plasticity. A relatively smaller and prolonged Ca^{2+} buildup results in LTD, while a larger, more transient increase favors LTP[6-12]. Examination of Ca^{2+} signals within CA1 dendritic spines demonstrates that synaptic stimulation results in NMDAr-mediated increases in Ca^{2+} that are limited to the spine head housing the activated synapse[13]. At resting potentials, NMDAr are largely blocked by extracellular Mg^{2+} ions[14]. The apparent affinity of Mg^{2+} for the receptor is strongly voltage-dependent and Ca^{2+} influx is greatly accentuated by depolarization. Thus membrane potential exerts a strong influence on spine Ca^{2+}

transients which influences downstream Ca^{2+}-dependent processes such as the induction of synaptic plasticity.

Here we show that SK channels are localized to dendritic spines and play a role in shaping synaptic responses. Apamin-sensitive SK channels are activated by synaptically-evoked Ca^{2+} transients and act to reduce the magnitude of excitatory postsynaptic potentials (EPSPs) measured at the soma. This negative regulation occurs within individual spines where SK channels respond to rapid increases in Ca^{2+} and reduce the amplitude of NMDAr-mediated Ca^{2+} transients. The effects of different Ca^{2+} buffers show that SK channels are positioned within a synaptically-activated Ca^{2+} signaling microdomain and act rapidly to influence the EPSP.

RESULTS

SK channel distribution in cultured hippocampal neurons

Mouse SK2 channels harboring an extracellular triple-myc epitope tag (mSK2-myc)[15] were expressed along with green fluorescent protein (GFP) in dissociated, cultured neurons of CA1 area of hippocampus. Live-cell immunostaining with a monoclonal anti-myc antibody performed one week after transfection revealed that virtually every spine was clearly decorated with SK2 channels (Fig. 1). Immunoreactivity was also present in clusters within the dendritic shafts and in the soma. Therefore, exogenously expressed SK2 channels localize to dendritic spines where they are positioned along with NMDAr in the confined Ca^{2+} signaling domain of glutamatergic synapses.

Apamin increases synaptically evoked EPSPs

The effects of apamin on subthreshold EPSPs recorded from whole-cell current-clamped CA1 neurons in acute slices from mouse hippocampus were determined. Following whole cell formation, a 100 μs current pulse was applied to the stratum radiatum and the stimulus strength was adjusted to ~one-third of the threshold for evoking an action potential, yielding EPSPs with amplitudes of 2 to 8 mV. Blocking NMDA receptors with dl-2-amino-5-phosphono-valeric acid (AP5; 100 μM) reduced subthreshold EPSP amplitudes to an average of 76 ± 3.8% of control, and co-application of AP5 with 6-Cyano-7-nitroquinoxaline-2, 3-dione (CNQX; 20 μM) to block AMPA receptors (AMPAr) essentially eliminated the EPSPs, reducing the average amplitude to

4.7 ± 0.6% of control (n = 3; not shown). These data show that although the EPSP results primarily from activation of AMPAr, a small but measurable contribution is made by current flow through NMDAr.

To determine whether apamin-sensitive SK channels shape the synaptic potential, EPSPs were recorded every 20 seconds in control solution and following wash-in of apamin (100 nM) (Fig. 2). In the representative cell in Figure 2a and 2b, the peak EPSP, measured from the average of 20 EPSPs increased from 3.3 mV in control solution to 5.6 mV following apamin application. Subsequent application of AP5 (100 μM) reversed the apamin-induced increase of the EPSP amplitude, so that in the presence of apamin and AP5 the peak of the averaged EPSP was 2.5 mV. On average, apamin increased the amplitude of the EPSP to 173 ± 16 % of control ($p < 0.05$, n = 5) and subsequent co-application of AP5 reduced the EPSP amplitude to 79 ± 13 % of control levels (Fig. 2c, Table 1). The apamin effect was observed in every cell tested.

SK channels are the only known targets for apamin, but apamin does not reliably wash out of the slice. Therefore, the effects of d-tubocurarine (100 μM)[16], a more reversible, although less selective SK channel blocker, were also examined. In 6 cells tested dTC increased the amplitude of the EPSP to 216 ± 53% ($p < 0.05$) of control. The increase induced by dTC was largely, but not completely reversed upon washout (154 ± 21% of control; $p = 0.05$; Table 1).

The induction of long-term potentiation usually requires repetitive synaptic activation and apamin shifts the threshold for the induction of synaptic plasticity to lower stimulus frequencies[4, 17]. Therefore, the effect of apamin on the summated EPSP during a synaptically evoked train of 5 pulses at 100 Hz was examined. Figure 2d shows a

representative cell in which apamin increased the average summated EPSP by 4 mV to 153% of control. Across 5 cells tested apamin application increased the summated EPSP to 159 ± 15% of control ($p < 0.05$; Fig. 2e).

NMDA receptor activity is required for apamin sensitivity of EPSPs

Dendritic spines are autonomous Ca^{2+} signaling compartments highly specialized for the rapid large-amplitude Ca^{2+} fluctuations that underlie synaptic plasticity[13]. The results presented above suggest that the effects of apamin on the amplitude of the EPSP are due to regulation of NMDAr in response to synaptically evoked increases in Ca^{2+}. Therefore, applying NMDAr blockers such as AP5 before apamin should occlude the effects of apamin. Indeed, blocking NMDAr with AP5 reduced the EPSP to 78 ± 5% of control ($p < 0.05$) and subsequent co-application of AP5 and apamin had no effect on the EPSP amplitude (96 ± 3% of the value in AP5, $n = 7$; Fig. 3a, b; Table 1).

SK channels are activated by changes in intracellular Ca^{2+} suggesting that augmenting synaptically evoked Ca^{2+} influx should increase SK channel activation and amplify the effect of apamin on the EPSP. Since ~80% of the Ca^{2+} transient in dendritic spines is due to Ca^{2+} influx through NMDA receptors (see Fig. 6), reducing Mg^{2+} block of NMDAr by lowering external Mg^{2+} to 0.2 mM should result in larger synaptically evoked Ca^{2+} influx. Under these conditions, apamin application increased the EPSP to 266 ± 29% of control ($p < 0.05$, $n = 6$) that was more than the apamin-induced increase when external Mg^{2+} was 1 mM ($p < 0.05$; Fig. 3c, d; Table 1). Preincubation with AP5 occluded the apamin-induced change in the EPSP in 0.2 mM Mg^{2+} (not shown).

The effects of apamin on the isolated NMDAr component of the response were

examined by recording EPSPs in the presence of CNQX (20 μM) or NBQX (10 μM) to block AMPA receptors (Fig. 3e, f). In initial experiments, setting the stimulus strength in control conditions to 1/3 of threshold compromised the ability of the stimulus to reliably elicit an EPSP after addition of AMPAr blockers, although subsequent application of apamin revealed an EPSP that was eliminated by AP5 (Fig. 3e). To resolve an NMDAr component, the stimulus strength was increased in AMPAr blockers to yield a detectable EPSP. Apamin increased the NMDAr-mediated EPSP to 320 ± 52 % of that in CNQX ($n = 9$, $p < 0.05$), and subsequent application of AP5 to five of the cells essentially eliminated the EPSP (0.3 ± 0.1 of control; Fig. 3e, f; Table 1).

Apamin increases EPSPs by a postsynaptic mechanism

The model presented above suggests that apamin acts post-synaptically, but bath application of apamin may have both pre- and post-synaptic effects. Paired-pulse facilitation (PPF), defined as the ratio of the amplitude of the second EPSP to that of the first following a closely delivered pair of stimuli, is a sensitive assay of presynaptic changes in glutamate release probability[18]. Using paired pulse stimulations, separated by 100 ms, apamin application increased the peaks of the EPSPs without altering PPF (2.47 ± 0.47 in control and 2.07 ± 0.19 in apamin, $n = 6$; Fig. 4a, b). This result is consistent with a postsynaptic site of action of apamin.

Apamin increases synaptically-evoked spine Ca^{2+} influx

Taken together, our results suggest that SK channels reside in the dendritic spine head, close to synaptically-evoked Ca^{2+} sources. If opening SK channels imposes a

repolarization that favors Mg^{2+} block of NMDA receptors, then blocking SK channels with apamin should increase the amplitude and prolong the duration of NMDAr-mediated Ca^{2+} influx. To test this hypothesis, spine Ca^{2+} transients were examined with 2-photon laser-scanning microscopy (2PLSM) while the synapse on the visualized spine was stimulated with 2-photon laser uncaging (2PLU) of MNI-glutamate (Fig. 5)[19]. 2PLU allows the selective stimulation of the synapse on any visualized spine and because of the brief and focal release of glutamate, can be used to accurately mimic the time course of innate synaptic events in hippocampal pyramidal neurons[20]. Furthermore, 2PLU is advantageous over electrical stimulation of axonal fibers because it allows the true stimulation of a single synapse on the cell, avoiding stimulation of multiple synapses that may lead to secondary activation of active conductances, altering the EPSP amplitude and time course. Lastly, by bypassing the presynaptic terminal, failures of synaptic transmission are avoided and trial-to-trial variability is reduced, allowing population comparison of synaptic responses in control conditions and in the presence of apamin.

CA1 pyramidal neurons were filled with the Ca^{2+}-sensitive fluorophore Fluo-5F (300 μM) to record Ca^{2+} transients and the Ca^{2+}-independent fluorophore Alexa-Fluor 594 (10 μM) to visualize spines and dendrites. Both fluorophores are efficiently 2-photon excited by 810 nm laser light with Fluo-5F emitting in the green (500-560 nm) and Alexa-Fluor 594 emitting in the red (585-630 nm). Under these conditions, changes in green fluorescence relative to resting red fluorescence ($\Delta G/R$) are linearly proportional to increases in intracellular [Ca^{2+}] following subthreshold synaptic stimulation[13].

Spines within the proximal (< 150 μm) apical dendrite were selected for analysis. Cells were initially held in voltage clamp and the uncaging laser pulse duration and

amplitude were set to evoke a ~15 pA synaptic current (uEPSC; Fig. 5b top, Table 2). The amplifier was subsequently switched to current-clamp mode and the uncaging evoked EPSP (uEPSP) and synaptically-evoked Ca^{2+} transient were monitored with the same uncaging parameters (Fig. 5b middle and bottom). The fluorescence transient that accompanies the uEPSP was recorded in line scan mode (500 Hz) from the stimulated spine head ($\Delta G/R_{syn}$) and the neighboring parent dendrite. In basal conditions, the uEPSP had an amplitude of 0.92 ± 0.09 mV, a 20-80% rise time of 2.8 ± 0.3 and a half-width of 30 ± 3 ms (properties of the uEPSC, uEPSP are summarized in Table 2). The uEPSP was accompanied by an increase in spine Ca^{2+} levels ($\Delta G/R_{syn}$ = 7.1 ± 0.8%) that rose quickly (20-80% risetime = 13 ± 2.0 ms) and was limited to the stimulated spine. Following stimulation, Ca^{2+} remains elevated in the spine head due to the slow clearance of Ca^{2+} from the spine in the presence of Ca^{2+} indicator[13]. To confirm that the evoked Ca^{2+} influx was due to activation of NMDAr, the same experiments were repeated in the presence of the NMDAr antagonists 2-carboxypiperazin-4-yl-propyl-1-1phosphonic acid (CPP, 20 μM) and MK801 (40 μM) (Figure 5c). In these conditions, the uEPSC and uEPSP were unchanged from control conditions (Table 2) whereas the amplitude of synaptically evoked Ca^{2+} transients was reduced to 1.3 ± 0.3% $\Delta G/R_{syn}$ (17% of control levels). Thus Ca^{2+} influx through NMDAr contributes the majority of the Ca^{2+} evoked by uEPSPs.

The effect of activation of SK channels on the amplitude of the Ca^{2+} transients within individual spine heads that contain active synapses was examined in a population of cells exposed to apamin (100 nM). The properties of the uEPSC were unaffected and uEPSP was slightly increased (Figure 6, Table 2). The smaller effect on synaptic potentials seen here is likely due to the reduced SK channel activation in the presence of

the BAPTA-based indicator Fluo-5F and the large variability of single-synapse evoked potentials (coefficient of variation of ~ 50%). The amplitude and the rising phase of uEPSP-evoked Ca^{2+} influx in the presence of apamin were increased to 12.2 ± 1.5% $\Delta G/R_{syn}$ and 23 ± 2 ms or 172% and 169% of control levels, respectively. The effects of SK channel blockade require active NMDAr since, in the presence of CPP + MK801, the uEPSC, uEPSP, and $\Delta G/R_{syn}$ were identical in control conditions and with apamin present (Figure 6b). Thus following activation of a single synapse SK channels act within the active spine to reduce the amplitude of NMDAr-mediated Ca^{2+} transients.

SK channels are located within a microdomain of synaptically-evoked Ca^{2+} sources

Our results indicated SK channels are located on the spine and in response to synaptically activated Ca^{2+} sources, regulate Ca^{2+} influx into the active spine head. To estimate the distance between SK channels and the Ca^{2+} source that activates them, synaptically evoked EPSPs were recorded with BAPTA, a rapid Ca^{2+} chelator, or EGTA, a slower Ca^{2+} chelator, in the internal solution. When the neuron was dialyzed with 5 mM BAPTA, the effects of apamin on the EPSP amplitude were occluded (89 ± 3% of control, n = 6; Fig. 7a, b, Table 1). In contrast, when 5 mM EGTA was included in the pipette solution application of apamin increased the EPSP to 214 ± 32% of control (n = 8, $p < 0.05$; Fig. 7c, d, Table 1) that was not different than the increase induced by apamin in control internal solution. Additionally, with 1 mM BAPTA in the pipette solution the apamin-mediated increases of the EPSP were 140 ± 11% of control ($p < 0.05$, n = 5; Table 1). These results further support a postsynaptic action by apamin and, importantly, are consistent with a close spatial coupling between SK channels and synaptically-

activated Ca^{2+} sources within individual spine heads.

DISCUSSION

The central finding of this work is that in dendritic spines of CA1 neurons, Ca^{2+} entry following synaptic activation opens SK channels that act to limit the amplitude of synaptic potentials and reduce Ca^{2+} influx through NMDAr. As described below, this feedback loop is engaged by the activity of a single synapse and regulates NMDAr-evoked Ca^{2+} influx within the active spine head. Since NMDAr-mediated Ca^{2+} is quantitatively critical to the processes underlying the induction of synaptic plasticity[6-12], this feedback loop likely accounts for the effects of apamin on the threshold for LTP induction. Furthermore, our results suggest that SK channel modulation offers a powerful mechanism to fine-tune the induction of synaptic plasticity.

Effects of apamin of synaptic responses

Blocking SK channels with apamin increased the synaptically-evoked EPSP amplitude. This effect was prevented by pretreatment with NMDAr blockers and was still present when the NMDAr component of the EPSP was isolated. Thus NMDAr are necessary and sufficient for the effects of apamin on the EPSP and apamin increases synaptic potentials by enhancing the normally small contribution of the NMDAr to the EPSP. The large effect that apamin has on NMDAr activation was particularly evident when the isolated NMDAr component of the EPSP was examined. Despite the small, or in several cells undetectable NMDAr-mediated response to synaptic stimulation, apamin application revealed a prominent AP5-sensitive EPSP. Since NMDAr contribute the majority of the uncaging-evoked Ca^{2+} influx into the spine head, their increased

activation in the presence of apamin boosts spine head Ca^{2+} transients by 72%. The effects of apamin on spine Ca^{2+} transients were, by necessity, monitored in cells loaded with Ca^{2+} indicators that will reduce the amplitude of evoked Ca^{2+} transients and subsequent activation of SK channels. Therefore, apamin application is likely to increase synaptically-evoked spine Ca^{2+} transients even more robustly in neurons free of BAPTA-based Ca^{2+}-buffers.

Apamin also increased the initial slope of the EPSP to an extent similar to that seen for the amplitude (Table 1). The initial slope was measured as the maximum rate of rise of the EPSP, which usually occurred between 10 and 20% of the rise time (~2 ms into the EPSP). The initial slope was increased for both the rapidly rising, mixed AMPAr and NMDAr-mediated EPSP and the slower, isolated NMDAr-mediated EPSP which had 20-80% rise times of 5.0 ± 0.1 ms and 16.1 ± 4.5 ms, respectively. Is the effect of apamin on the rate of rise of the EPSP compatible with the slow opening of NMDAr? The macroscopic kinetics of the synaptically evoked NMDAr mediated component of the EPSP, which reaches a peak in about 20 ms[21,22], has been attributed to the intrinsic gating kinetics of the channel[23, 24]. Complementary single channel records show that gating is complicated, but heterologously expressed NMDA receptors activate with 10-90% rise times of ~7-11 ms[25,26]. The predominant component of Mg^{2+} unblock of NMDAr is very fast, occurring in ~100 μs[27], and is not rate-limiting. Taken together, these data suggest that at least a small number of NMDA receptors open early in the response and contribute to the rising phase and peak of the EPSP. These early-opening channels may also provide the source of Ca^{2+} that rapidly activates apamin-sensitive SK channels.

Regulation of synaptic responses by SK channels

Our data indicates that SK channels are located near glutamatergic synapses in dendritic spines. We demonstrate that exogenously expressed SK2 channels are targeted to spine heads and that activation of a single synapse engages SK channels to regulate NMDAr-dependent Ca^{2+} influx within the active spine head. What synaptically evoked Ca^{2+} sources open SK channels? Our results demonstrate that ~80% of the spine head Ca^{2+} influx is contributed by NMDAr and the remaining 20% likely reflects activation of voltage-sensitive Ca^{2+} channels, which in spines of hippocampal pyramidal neurons are R-type[28,29]. Thus, rapid Ca^{2+} influx through NMDAr that open in the early portion of the EPSP (see above) with a possible contribution from voltage-sensitive Ca^{2+} channels is likely to provide the signal that activates SK channels.

The close association between synaptically-activated Ca^{2+} sources and SK channels is supported by results showing that 5 mM BAPTA, a rapid Ca^{2+} chelator, is capable of intercepting the Ca^{2+} ions before they encounter SK channels, while the slower Ca^{2+} chelator, EGTA at the same concentration, does not block the effect. The characteristic distance for the diffusion of Ca^{2+} in 3 dimensions before capture by a buffer can be approximated by $\sqrt{6 \bullet D_{Ca} \bullet k_{on} \bullet [buffer]}$ where D_{Ca} is the diffusion coefficient of Ca^{2+}, k_{on} is the apparent forward rate constant of Ca^{2+} binding to the buffer and [buffer] is the free buffer concentration. Using a k_{on} of 4×10^8 $M^{-1}sec^{-1}$ for BAPTA and a D_{Ca} of 220 $\mu m^2 sec^{-1}$ (ref. 30), Ca^{2+} will diffuse a characteristic distance of 26 nm before capture by 5 mM BAPTA and 58 nm before capture by 1 mM BAPTA. Therefore, the distance between the two populations of channels may be estimated at ~25-50 nm and Ca^{2+} ions will encounter an SK channel within ~2 μs of entering the spine.

Since subthreshold synaptic stimulation increases average Ca^{2+} levels within the spine head to approximately 700 nM[13], activated Ca^{2+} sources are likely to locally provide a saturating concentration (> 3 μM) of Ca^{2+} to the SK channels, so that the intrinsic gating kinetics of the SK channels will influence the EPSP. Rapid application of saturating concentrations of Ca^{2+} to heterologously expressed SK channels shows that activation time constants can be < 5 ms[31]; (unpublished results, J.M., J.P.A.), consistent with their influence on the initial slope of the EPSP. In an earlier study, Marrion and Tavalin examined the coupling between single L-type Ca^{2+} channels and SK channels on the soma of CA1 neurons[32,33] and demonstrated latencies between L-type channel and SK channel opening as short as 0.9 ms and channel separation of ~100 nm[34].

Our results are consistent with previous demonstrations of coupling between NMDA receptors and Ca^{2+}-activated K^+ channels on cultured hippocampal neurons. Zorumski et al, showed that application of NMDA to cultured postnatal rat hippocampal neurons evoked a Ca^{2+}-activated K^+ current that was partially inhibited by d-TC but was not blocked by apamin[35]. More recently, Shah and Haylett applied NMDA to the soma of cultured hippocampal neurons and observed activation of an SK current in ~0.5 of the cells. Their data also showed that the muscarine-sensitive, slowly activating Ca^{2+}-activated K^+ current was sometimes activated by NMDA application, but they did not observe coupling to large conductance Ca^{2+}-activated BK channels[36], as reported for olfactory neurons[37]. In dopamine neurons, NMDA receptor activation may decrease an associated SK current[38].

SK2 channels are the subtype that is likely responsible for the apamin-induced effects on the EPSPs, the Ca^{2+} transients, and synaptic plasticity. A recent study using SK

knockout mice showed that SK2 channels account for the apamin-sensitive currents in CA1 neurons[39]. Consistent with this, we demonstrate that immunohistochemistry of SK2-transfected CA1 neuronal cultures detected SK2 channels in virtually every spine. In addition, SK2 channels are seen in within the dendritic shafts, where SK channel activation affects plateau potentials and the spread of dendritic Ca^{2+} spikes by influencing voltage-gated Ca^{2+} channels[40]. SK2 immunoreactivity is also observed throughout the soma and this population may mediate the apamin-induced increase of CA1 excitability[4,34,41]. Therefore, separate populations of SK2 channels in CA1 neurons occupy discrete subcellular locales where they couple to different Ca^{2+} sources to serve distinct physiological roles.

MATERIAL AND METHODS

Cell culture and transfection Primary cultures were prepared from the CA1 area of hippocampi of P1-2 mice as described previously[42]. Briefly, CA1 area from newborn mice were dissociated by papain treatment and trituration and plated on a bed of astrocytes at a density of ~7100 cells/cm^2. Neurons were cultured in MEM and supplemented with 5% horse serum. After 7 to 14 day in culture, cells were transfected with Lipofectamine2000 (InVitrogen, Carlsbad, CA). Immunocytochemistry was performed 5-7 days after transfection. At this time the neurons have branching dendritic arbors and well developed spines.

Immunocytochemistry To detect triple-myc labeled mSK2 protein on the cell surface, live cells were incubated with anti-myc mouse monoclonal antibody (Invitrogen, Carlsbad, CA) for one hour (37°C, 5% CO_2), washed, fixed, and permeabilized in 4% paraformaldehyde/4% sucrose in PBS for 30 min at room temperature and washed again. Neurons were pre-incubated in blocking solution (5% NGS/1% BSA) for 30 min at room temperature, and then incubated for 1 hour in Cy3-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA). Samples were mounted with Antifade mounting medium prior to visualization. Imaging was performed using a Leica DM-RXA microscope equipped with Zeiss Plan-Neofluor 40X oil NA1.4 and 63X oil NA1.25 objective lenses and a Princeton Instruments Micromax CCD camera. Images were acquired via MetaMorph acquisition and analysis software.

Slice preparation. All procedures were done in accordance with the guidelines of Oregon Health & Science University and Harvard University. Hippocampal slices were

prepared from C57BL/6J mice from postnatal day 15-18 for imaging/uncaging experiments and from postnatal week 4-6 for electrophysiology experiments. Animals were anesthetized by intraperitoneal injection with either a ketamine/xylocaine cocktail or isoflurane, and decapitated. The cerebral hemispheres were quickly removed and placed into cold artificial CSF and equilibrated with 95%O_2/5%CO_2. Hippocampi were removed, placed onto an agar block, and transferred into a slicing chamber containing sucrose-ACSF or choline-ACSF. Transverse hippocampal slices (300-375 μm) were cut with a Leica VT1000s (Leica Instruments, Nussloch, Germany) and transferred into a holding chamber containing regular ACSF (in mM): 125 NaCl, 2.5 KCl, 21.4 $NaHCO_3$, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 11.1 glucose and equilibrated with 95%O_2/5%CO_2. Slices were incubated at 35°C for 30-45 min and then for synaptically evoked responses allowed to recover at room temperature for ≥1.5 hrs before recordings were performed.

Electrophysiology. For synaptically evoked recordings, CA1 pyramidal cells were visualized with IR/DIC optics (Zeiss Axioskop 2FS) and a CCD camera (Sony, Tokyo, Japan). Whole-cell patch-clamp recordings were obtained from CA1 pyramidal cells using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA), digitized using an ITC-16 analog-to-digital converter (InstruTech, Port Washington, NY) and transferred to a computer using Pulse software (Heka Elektronik, Lambrecht/Pfalz, Germany). Patch pipettes (open pipette resistance, 2.5-3.5MΩ), if not otherwise noted, were filled with a solution containing (in mM): 140 $KMeSO_4$, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 5 MgATP, 0.05 EGTA (pH 7.3). Excitatory postsynaptic potentials (EPSPs) were recorded in whole-cell current-clamp mode. A bipolar tungsten electrode (FHC, Bowdoinham, ME) was used to stimulate presynaptic axons in stratum radiatum. Picrotoxin (0.1mM) was added

in most experiments to reduce GABAergic contributions. The input resistance was determined from a ~7 pA hyperpolarizing current injection pulse given 800 ms after each synaptically evoked EPSP. Subthreshold EPSPs were elicited by 100μs current injections that were ~1/3 of the stimulus required for evoking an action potential. In some experiments, the magnitude of the apamin-induced increase of the EPSP elicited action potentials. Under these conditions, the stimulus strength was reduced to ~1/4 of threshold. Recordings were made using an Axon 200A amplifier (Axon Instruments, Union City, CA) interfaced to a Macintosh G4 with an ITC-16 computer interface (Instrutech Corp, Port Washington, NY). Data was filtered at 5 kHz and collected at a sample frequency of 20 kHz using Pulse (Heka Elektronik, Lambrecht/Pfalz, Germany). All recordings used cells with a resting membrane potential <-60mV that did not change by more than 2 mV during an experiment and a stable input resistance that did not change by more than 5%.

Data analysis. Data were analyzed using IGOR (WaveMetrics, Lake Oswego, OR). The slope of the rising phase of the EPSP was determined from its first derivative and taken as the maximum value during the first half of the rising phase. Data are expressed as mean ± SEM. Paired t-tests or ANOVA was used to determine significance; $p < 0.05$ was considered significant.

Pharmacology. Apamin (Calbiochem, La Jolla, CA), DL-AP5 (Tocris Cookson, Ellisville, MO), CPP (Tocris Cookson, Ellisville, MO), and MK801 (Tocris Cookson, Ellisville, MO) were dissolved in distilled water, and CNQX, NBQX, and Picrotoxin (Tocris Cookson, Ellisville, MO) in ethanol to produce stock solutions (1000x concentrated) and added to the external medium.

2-photon uncaging and imaging. Combined 2-photon uncaging of MNI-glutamate and 2-photon intracellular Ca^{2+} imaging was performed using a modified, custom 2PLSM microscope43 that is described fully elsewhere[19]. Briefly, the outputs of two Ti:Sapphire lasers (Mira/Verdi, Coherent, Santa Clara, CA) were independently modulated with Pockel's cells (350-80 and 350-50, Conoptics, Danbury, CT) and combined using polarizing optics. Glutamate was uncaged using 0.2-0.6 ms pulses of 720 nm light whereas Alexa-Fluor 594 and Fluo-5F were excited with 810 nm light. Fluorescence was collected in line scan mode (500 Hz) with a brief interruption (2 ms) during which the scanning mirrors were redirected to the selected spine and the uncaging pulse was triggered. Fluorescence transients were quantified as increases in green (Fluo-5F) fluorescence from baseline divided by resting red (Alexa Fluor 594) fluorescence. This method provides quantification that is insensitive to small changes in resting Ca^{2+} and independent of spine volume[13]. In our recording conditions $\Delta G/R$ is linearly proportional to $\Delta[Ca^{2+}]$ to within ~ 20%[13]. Off-line data analysis was performed using custom software written in Igor Pro (Wavemetrics) and MATLAB.

MNI-glutamate was included in the bath at 5 mM. Cells were filled with 300 μM Fluo-5F to report intracellular Ca^{2+} transients and 10 μM Alexa Fluor-594 to image neuronal morphology. Neurons were allowed to fill for 10-20 minutes before beginning Ca^{2+} imaging. Spines on secondary and tertiary dendrites within 150 μm of the soma were selected for analysis. uEPSCs and uEPSPs were monitored with an Axopatch 200B and were filtered at 2 kHz and sampled at 10 kHz.

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Figures and legends

Figure 1

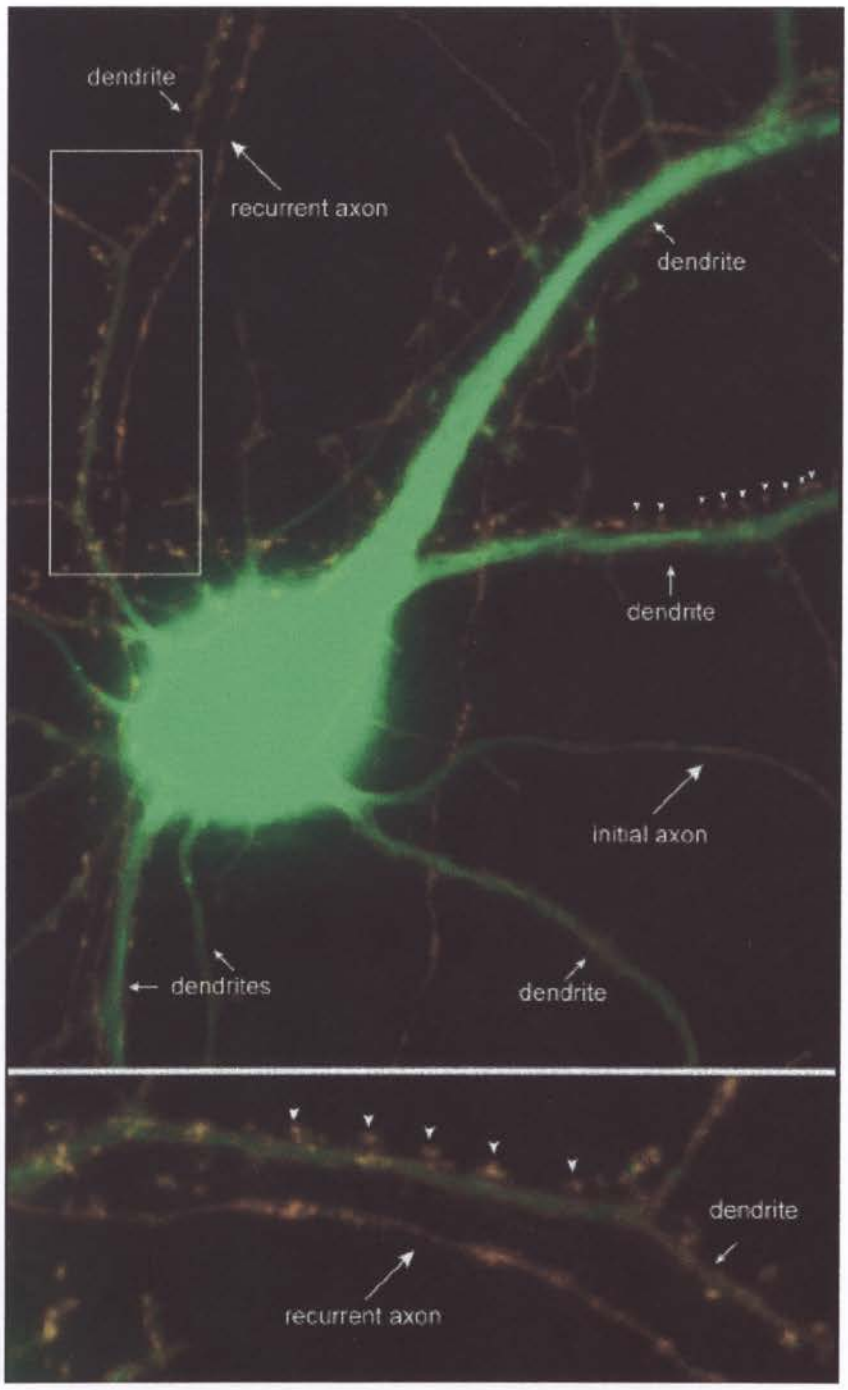
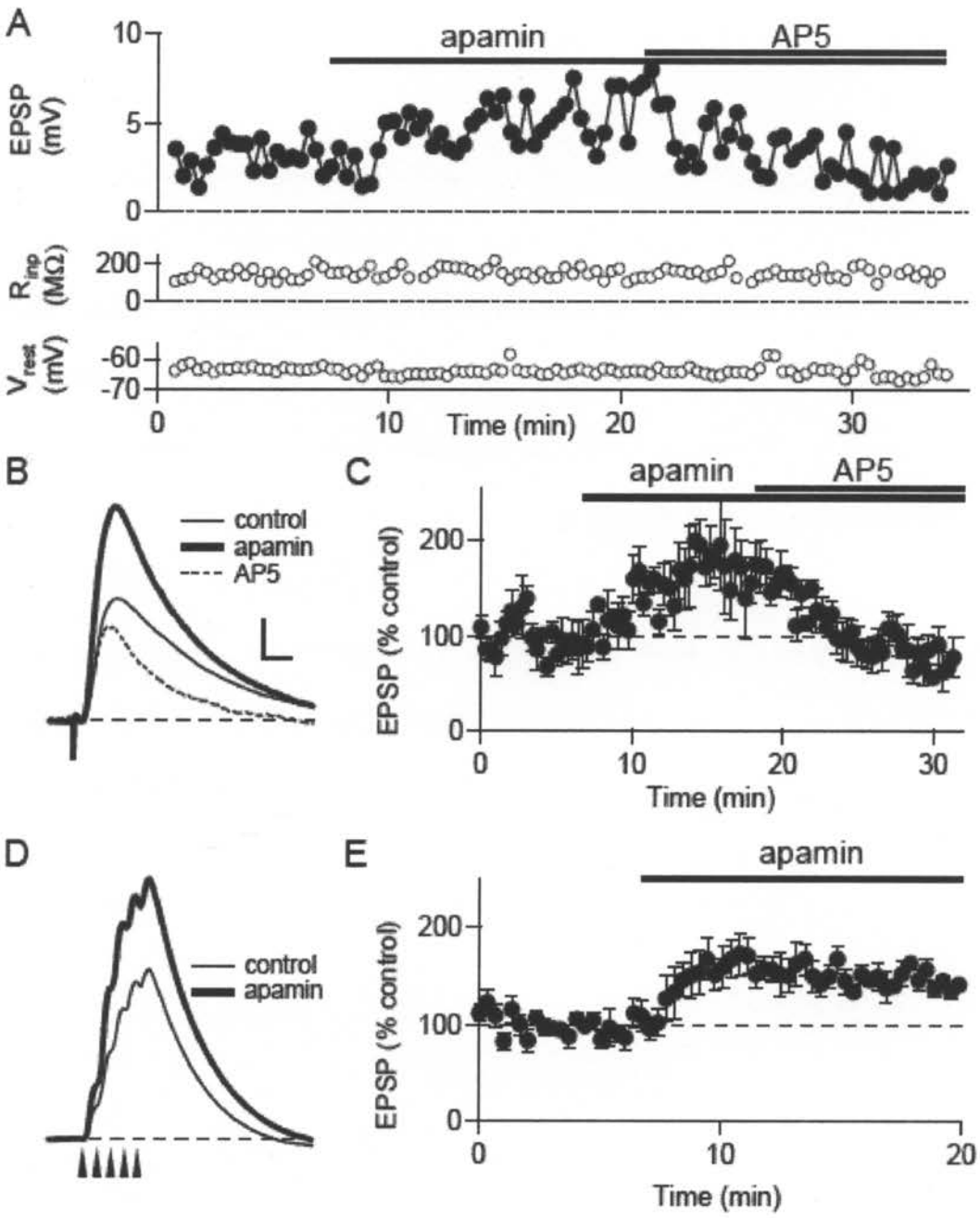


Figure 1 Live-cell immunostaining of a cultured hippocampal neuron expressing mSK2-myc (red) and cytosolic GFP (green).

SK2 channel immunoreactivity can be seen in virtually every spine. Clusters of SK2 immunoreactivity were detected in the dendritic shafts and a recurrent axon. Although obscured by the intensity of the GFP fluorescence, a more homogeneous SK2 staining pattern was detected in the soma.

Figure 2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apamin
AP5
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10
5
0
R_{inp} (MΩ)
200
0
V_{rest} (mV)
-60
-70
0
10
Time (min)
20
30
B
control
apamin
AP5
C
apamin
AP5
EPSP (% control)
200
100
0
0
10
20
30
Time (min)
D
control
apamin
E
apamin
EPSP (% control)
200
100
0
0
10
20
Time (min)

Figure 2 SK channels modulate of synaptically evoked EPSPs.

a, Diary plot of EPSP amplitude (top), membrane potential (middle), and input resistance (bottom) from a representative cell during wash in of apamin (100 nM) and apamin + AP5 (100μM).

b, Average of 20 EPSPs acquired in control conditions (thin trace), in the presence of apamin (thick trace), or in the presence of apamin + AP5 (dashed trace).

c, Summary plot of the EPSP amplitude relative to the baseline period during wash in of apamin and apamin + APV (n = 5 cells).

d, Average of 15 summated EPSPs in control conditions (thin trace) and in the presence of apamin (thick trace) from a representative cell. Arrowheads indicate stimulus times (5 pulses at 100 Hz).

e, Summary plot of the effects of apamin on the summated EPSP (n = 5 cells). The times of drug application are indicated by the horizontal bars. Scale bar: 1 mV in (b), 2 mV in (d), 25 ms for (b) and (d).

Figure 3

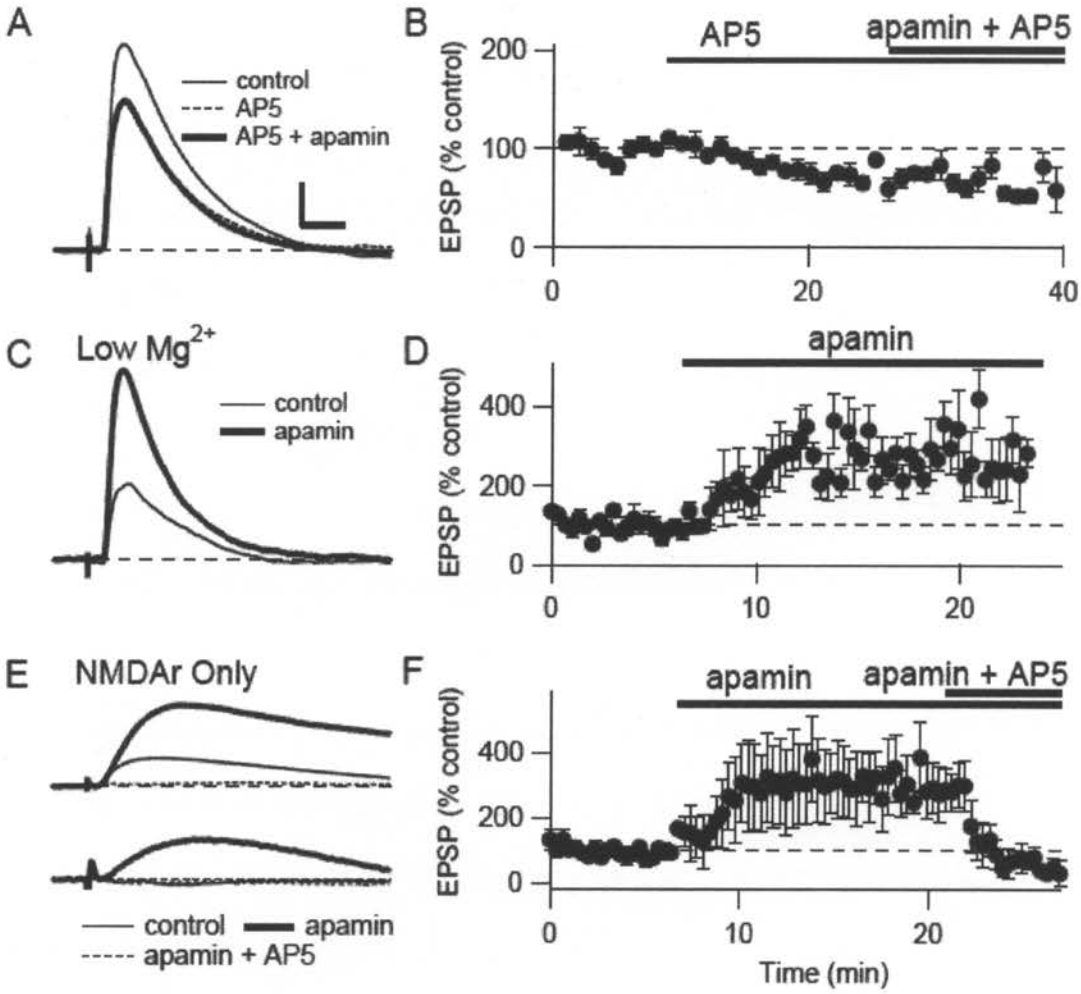

A
control
AP5
AP5 + apamin
B
AP5
apamin + AP5
EPSP (% control)
200
100
0
0
20
40
C
Low Mg2+
control
apamin
D
apamin
EPSP (% control)
400
200
0
0
10
20
E
NMDAr Only
control
apamin
apamin + AP5
F
apamin
apamin + AP5
EPSP (% control)
400
200
0
0
10
20
Time (min)

Figure 3 Apamin-induced EPSP increases require NMDAr activity.

a, Average of 25 EPSPs in control conditions (thin trace), in the presence of AP5 (dashed trace), or AP5 + apamin (thick trace) from a representative cell.

b, Summary plot of the effects of AP5 and AP5+apamin application on EPSP peaks (n = 7 cells).

c, Average EPSPs in 0.2 mM external Mg^{2+} (thin trace) or 0.2 mM Mg^{2+} + apamin (thick trace) containing solutions.

d, Summary plot of the effects of apamin application on EPSP peaks in low external Mg^{2+} (n = 6).

e, Average evoked EPSPs recorded in the presence of CNQX and 0 Mg^{2+} (control, thin trace) and with the additional application of apamin (thick trace) or AP5 + apamin (dashed trace). Examples are shown in which the NMDAr dependent EPSP was clearly visible (top) or not detectable (bottom) prior to apamin application.

f, Summary plot of the effects of apamin and apamin + AP5 on the peak of the isolated NMDAr-mediated EPSP (n = 5). The times of drug application are indicated by the horizontal bars. Scale bar: 1 mV for (a, c, and e), 0.5 mV for (f), and 25 ms for (a, c, e and f).

Figure 4

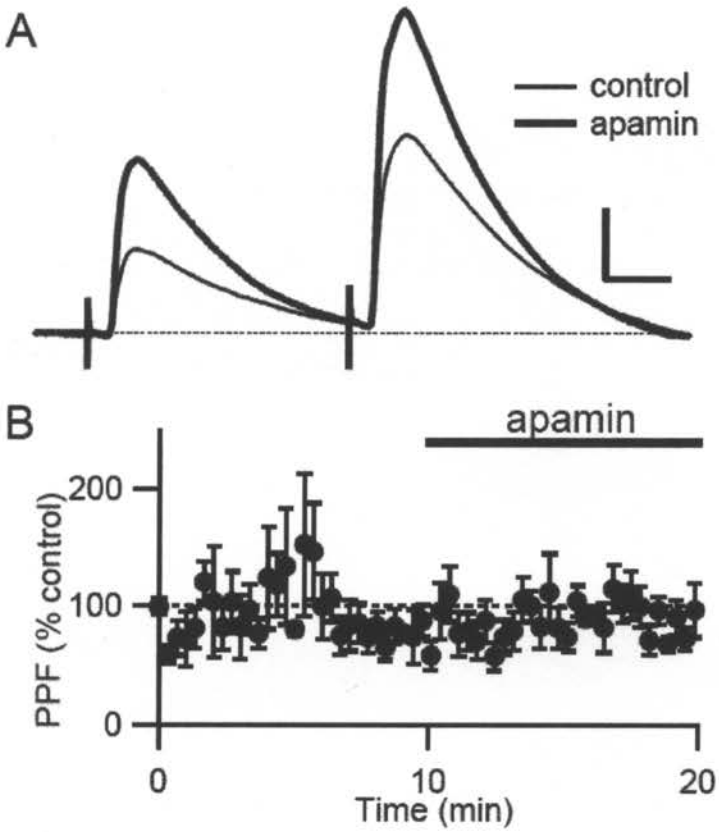

A
control
apamin
B
apamin
PPF (% control)
200
100
0
0
10
20
Time (min)

Figure 4 Apamin has no effect on paired pulse facilitation.

a, Synaptically-evoked EPSP pairs (100 ms interpulse interval) in control conditions (thin trace) and in the presence of apamin (thick trace) from a representative cell. Scale bar: 1 mV, 25 ms.

b, Summary plot of PPF relative to control values during application of apamin as indicated by the horizontal bar (n = 6 cells).

Figure 5

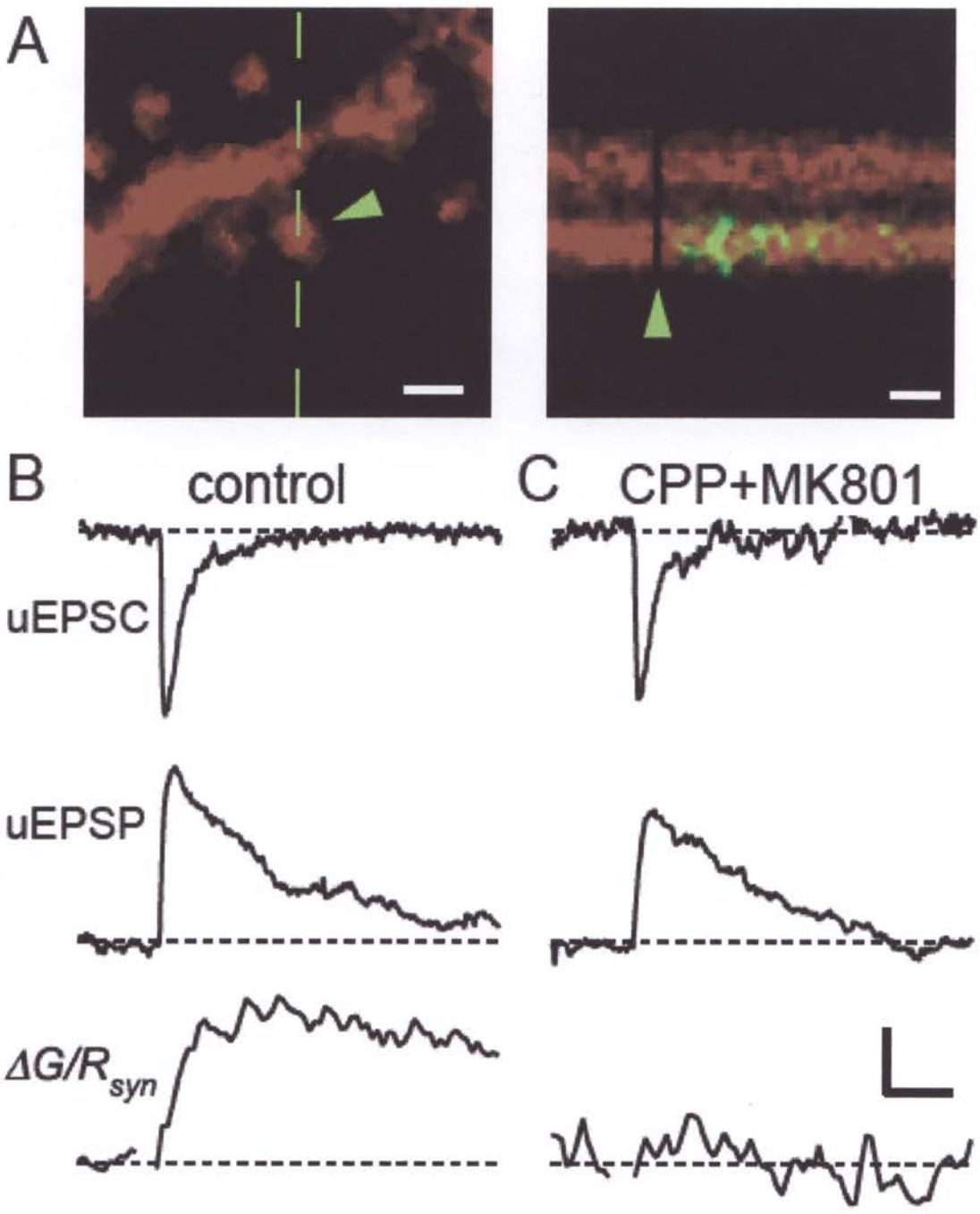


Figure 5 Single synapse responses and NMDAr-dependent spine Ca^{2+} signals evoked with 2-photon uncaging of glutamate.

a, Image of an apical spiny dendrite of a CA1 hippocampal pyramidal neuron collected with 2PLSM (left). Fluorescence from the red Alexa Fluor-594 channel is shown. Simultaneously recorded green (Fluo-5F) and red (Alexa Fluor-594) fluorescence (right) collected in line scans that intersect the spine head and neighboring dendrite as indicated by the dashed line in the left panel. The current was evoked by a 0.5 ms, 720 nm glutamate uncaging pulse directed at the spine indicated by the arrowhead. Increases in green fluorescence, indicative of elevations in intracellular Ca^{2+}, are limited to the stimulated spine head. Scale bar: 1 μm (right), 25ms (left).

b, For the spine depicted in panel (a), uncaging parameters were set to evoked an ~ 15 pA uEPSC (top). After switching to current clamp mode and leaving the uncaging pulse unchanged, the uEPSP (middle) and fluorescence transient (*ΔG/Rsyn*, bottom) were recorded.

c, Representative uEPSC (top), uEPSP (middle) and accompanying Ca^{2+} transient (bottom) recorded in the presence of NMDAr blockers (20 μM CPP and 40 μM MK801). Each trace is the average of 10 trials. Scale bar: 6 pA (top), 0.45 mV (middle), 3% ΔG/R (bottom), and 25 ms for panels (b) and (c).

Figure 6

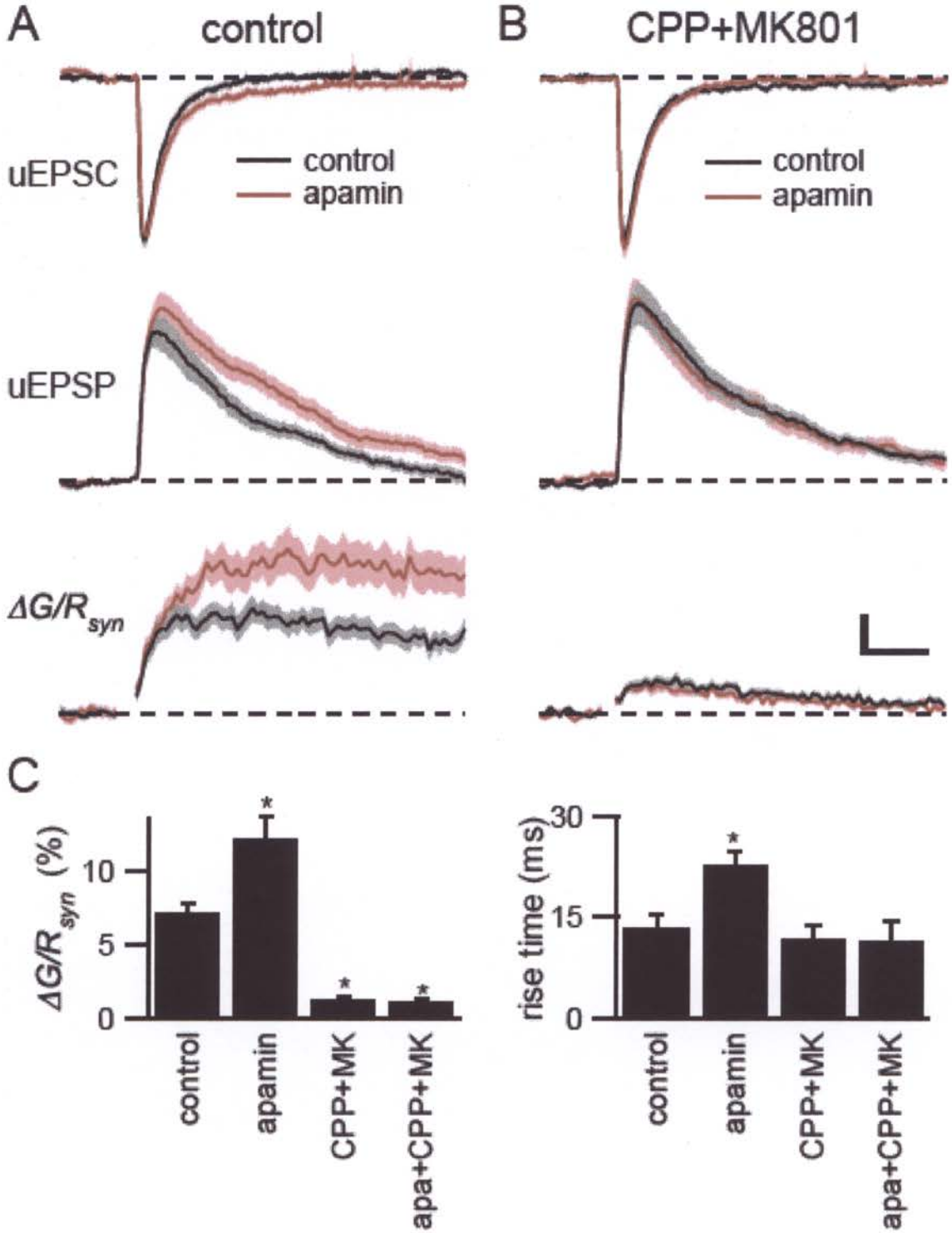

A
control
B
CPP+MK801
uEPSC
control
apamin
control
apamin
uEPSP
ΔG/R_syn
C
ΔG/R_syn (%)
10
5
0
control
apamin
CPP+MK
apa+CPP+MK
rise time (ms)
30
15
0
control
apamin
CPP+MK
apa+CPP+MK

Figure 6 Blocking SK channels with apamin increases NMDAr-mediated spine Ca^{2+} transients.

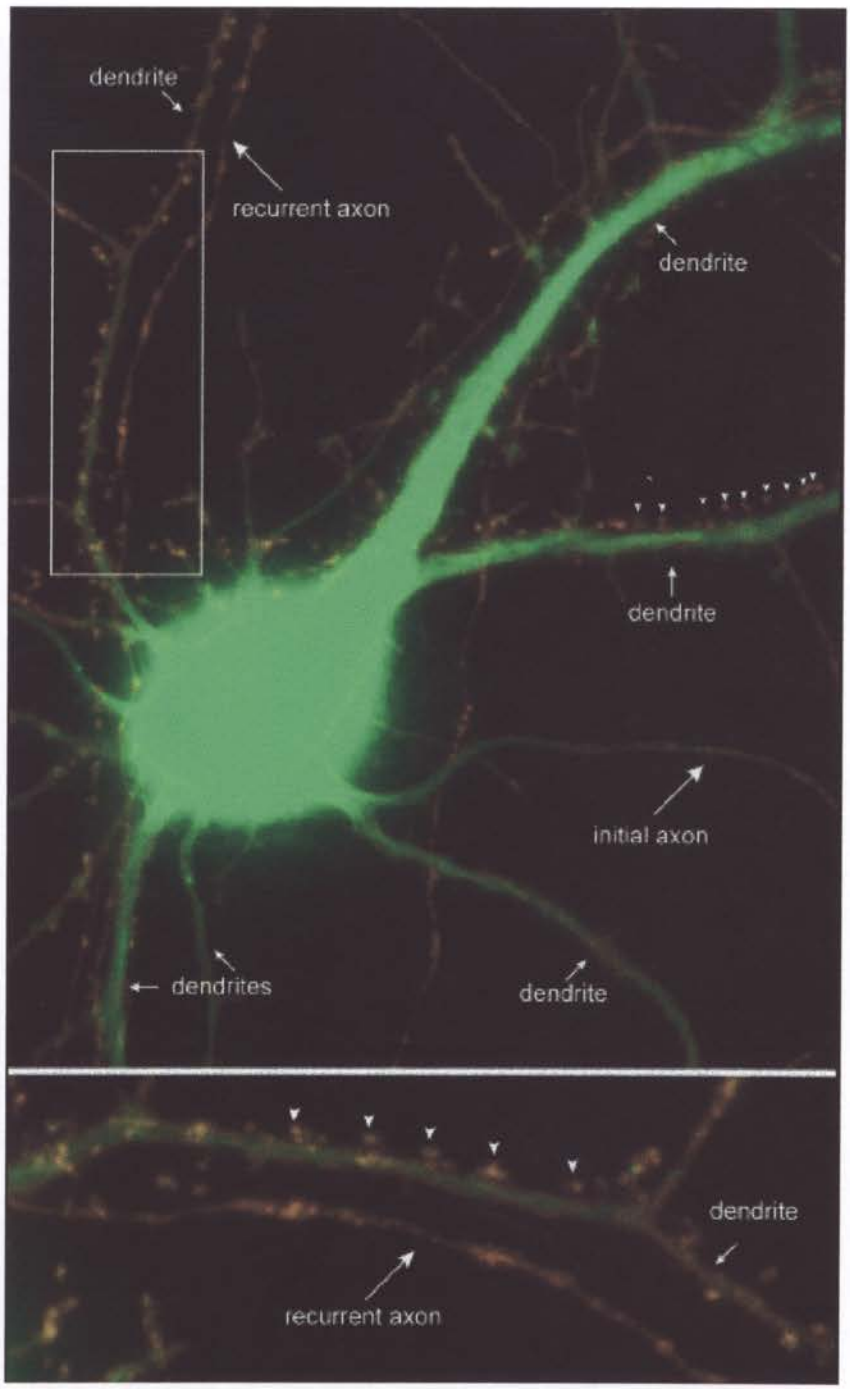
a, Average uEPSC (top), uEPSP (middle) and *ΔG/Rsyn* (bottom) in control conditions (black) and in the presence of apamin (red) (n = 24 and 25 spines respectively).

b, Average uEPSC (top), uEPSP (middle) and *ΔG/Rsyn* (bottom) in the presence of NMDAr blockers (black) and NMDAr blockers + apamin (red) (n = 21 and 22 spines respectively). Scale bar: 6 pA (top), 0.45 mV (middle) and 6% ΔG/R (bottom), and 25 ms for panels (a) and (b). The shaded region shows the mean ± SEM for each average trace.

c, Comparison of the Ca^{2+} transient amplitude and rise time each condition. * indicates $p < 0.05$ compared to control.

Figures and legends

Figure 1



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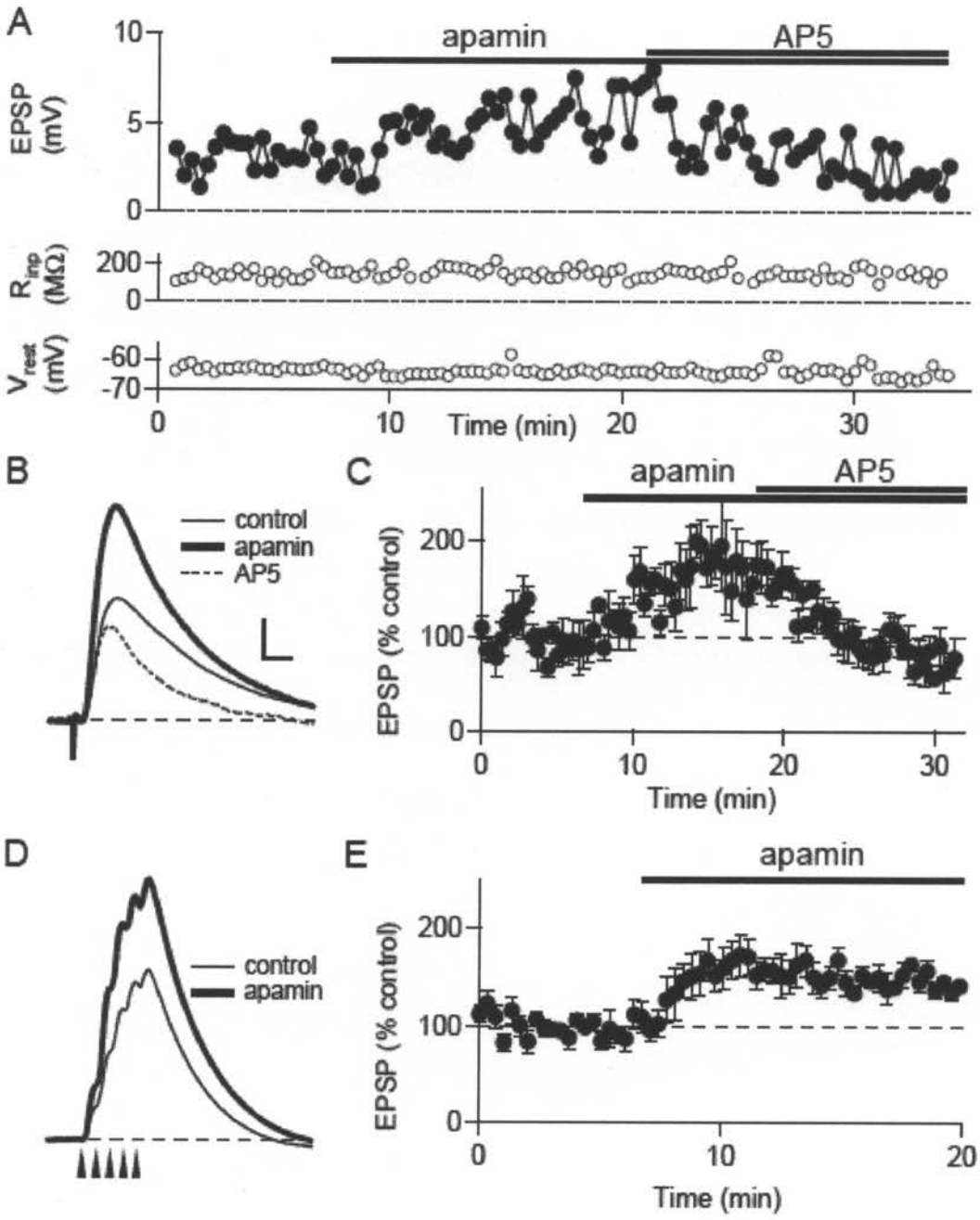
A
apamin
AP5
EPSP (mV)
10
5
0
R_{inp} (MΩ)
200
0
V_{rest} (mV)
-60
-70
0
10
Time (min)
20
30
B
control
apamin
AP5
C
apamin
AP5
EPSP (% control)
200
100
0
0
10
20
30
Time (min)
D
control
apamin
E
apamin
EPSP (% control)
200
100
0
0
10
20
Time (min)

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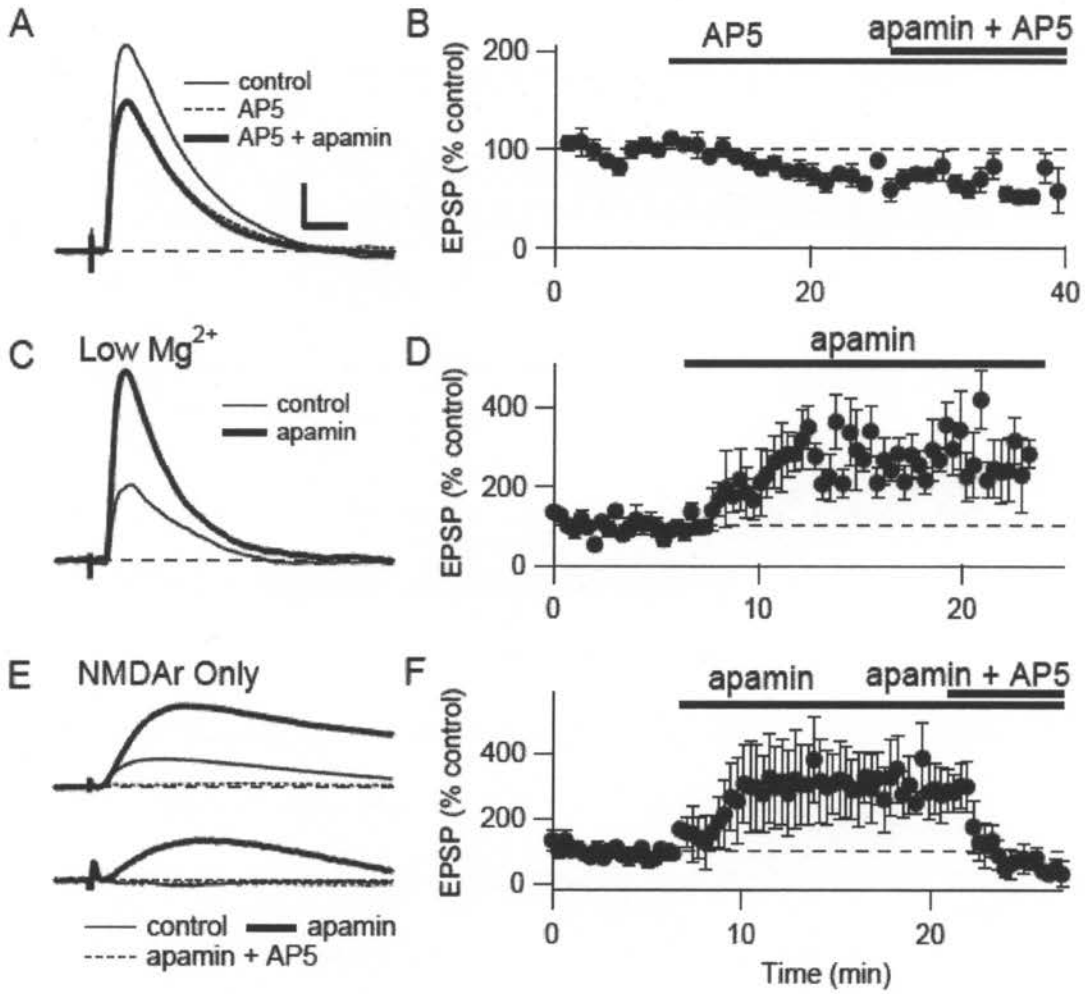
A
control
AP5
AP5 + apamin
B
AP5
apamin + AP5
EPSP (% control)
200
100
0
0
20
40
C
Low Mg2+
control
apamin
D
apamin
EPSP (% control)
400
200
0
0
10
20
E
NMDAr Only
control
apamin
apamin + AP5
F
apamin
apamin + AP5
EPSP (% control)
400
200
0
0
10
20
Time (min)

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f, Summary plot of the effects of apamin and apamin + AP5 on the peak of the isolated NMDAr-mediated EPSP (n = 5). The times of drug application are indicated by the horizontal bars. Scale bar: 1 mV for (a, c, and e), 0.5 mV for (f), and 25 ms for (a, c, e and f).

Figure 4

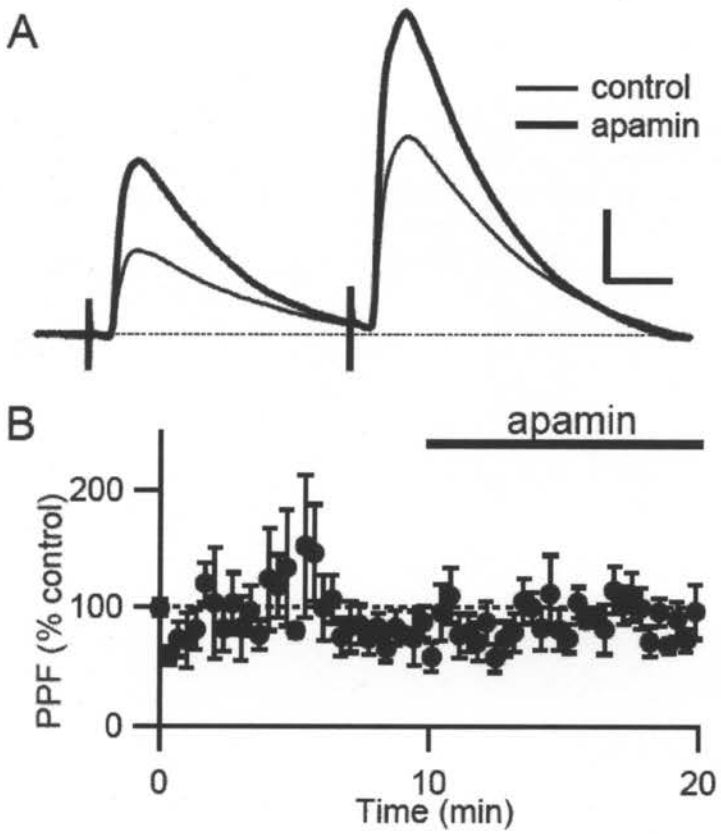

A
control
apamin
B
apamin
PPF (% control)
200
100
0
0
10
20
Time (min)

Figure 4 Apamin has no effect on paired pulse facilitation.

a, Synaptically-evoked EPSP pairs (100 ms interpulse interval) in control conditions (thin trace) and in the presence of apamin (thick trace) from a representative cell. Scale bar: 1 mV, 25 ms.

b, Summary plot of PPF relative to control values during application of apamin as indicated by the horizontal bar (n = 6 cells).

Figure 5

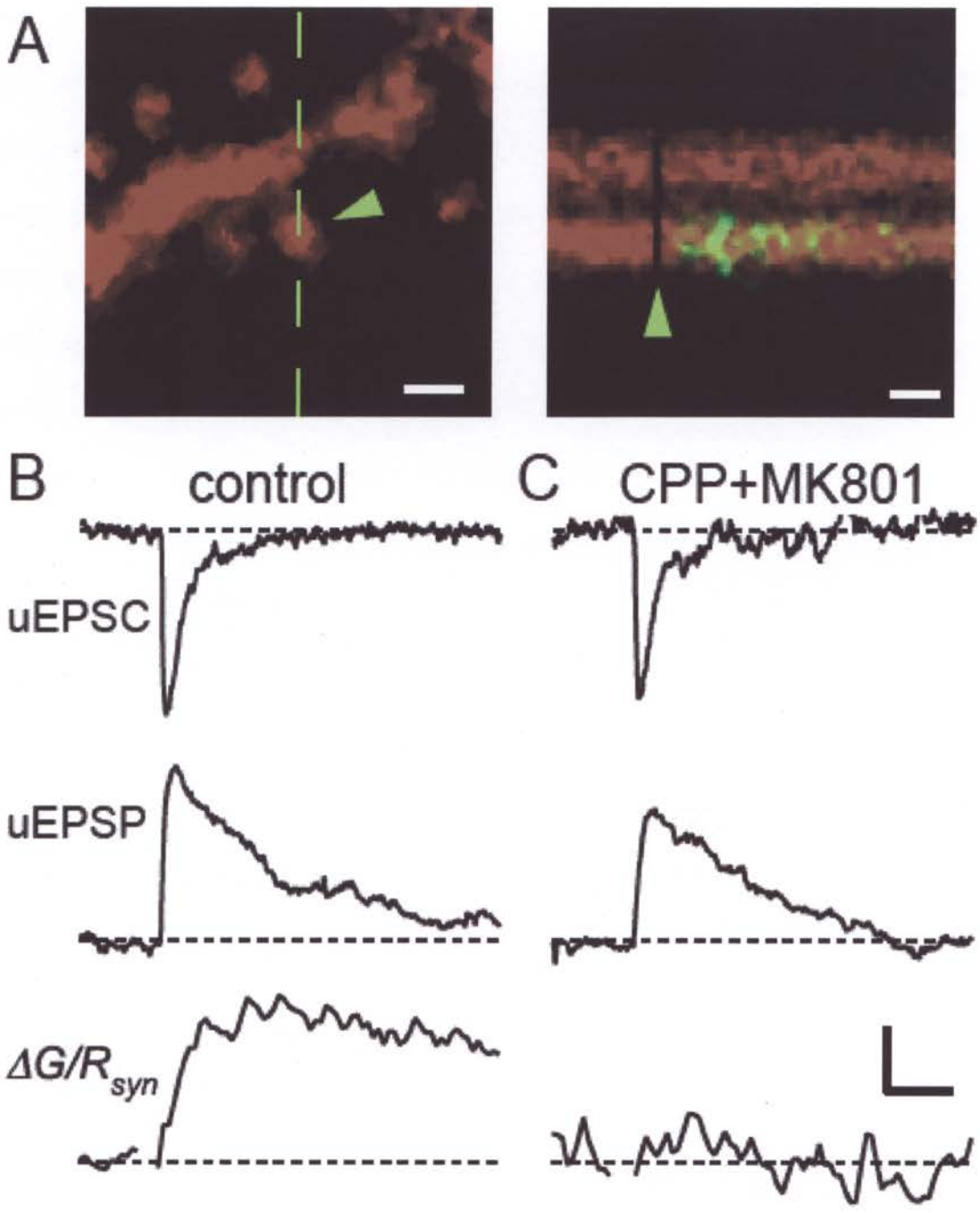


Figure 5 Single synapse responses and NMDAr-dependent spine Ca^{2+} signals evoked with 2-photon uncaging of glutamate.

a, Image of an apical spiny dendrite of a CA1 hippocampal pyramidal neuron collected with 2PLSM (left). Fluorescence from the red Alexa Fluor-594 channel is shown. Simultaneously recorded green (Fluo-5F) and red (Alexa Fluor-594) fluorescence (right) collected in line scans that intersect the spine head and neighboring dendrite as indicated by the dashed line in the left panel. The current was evoked by a 0.5 ms, 720 nm glutamate uncaging pulse directed at the spine indicated by the arrowhead. Increases in green fluorescence, indicative of elevations in intracellular Ca^{2+}, are limited to the stimulated spine head. Scale bar: 1 μm (right), 25ms (left).

b, For the spine depicted in panel (a), uncaging parameters were set to evoked an ~ 15 pA uEPSC (top). After switching to current clamp mode and leaving the uncaging pulse unchanged, the uEPSP (middle) and fluorescence transient (*ΔG/Rsyn*, bottom) were recorded.

c, Representative uEPSC (top), uEPSP (middle) and accompanying Ca^{2+} transient (bottom) recorded in the presence of NMDAr blockers (20 μM CPP and 40 μM MK801). Each trace is the average of 10 trials. Scale bar: 6 pA (top), 0.45 mV (middle), 3% ΔG/R (bottom), and 25 ms for panels (b) and (c).

Figure 6

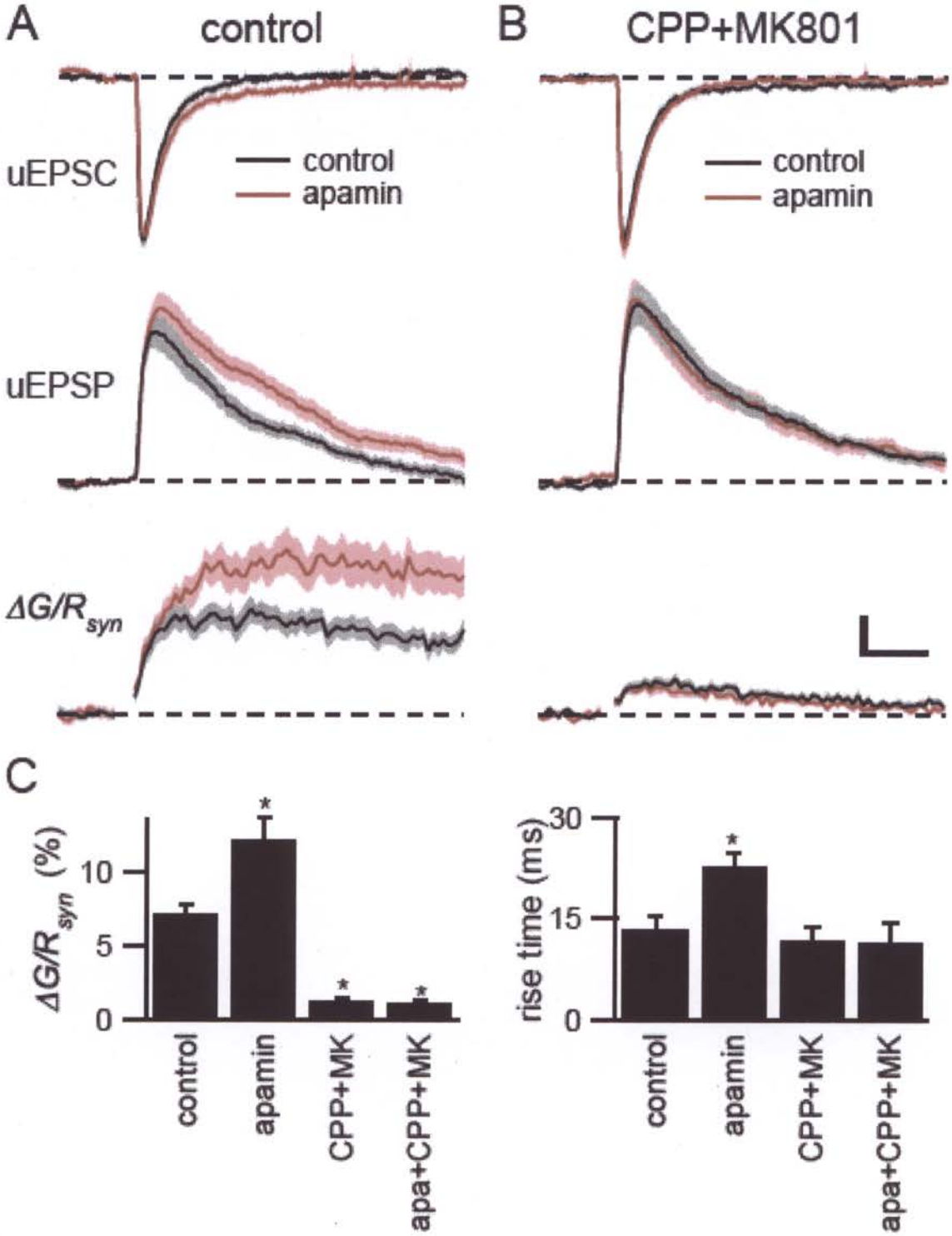

A
control
B
CPP+MK801
uEPSC
control
apamin
control
apamin
uEPSP
ΔG/R_syn
C
ΔG/R_syn (%)
10
5
0
control
apamin
CPP+MK
apa+CPP+MK
rise time (ms)
30
15
0
control
apamin
CPP+MK
apa+CPP+MK

Figure 6 Blocking SK channels with apamin increases NMDAr-mediated spine Ca^{2+} transients.

a, Average uEPSC (top), uEPSP (middle) and *ΔG/Rsyn* (bottom) in control conditions (black) and in the presence of apamin (red) (n = 24 and 25 spines respectively).

b, Average uEPSC (top), uEPSP (middle) and *ΔG/Rsyn* (bottom) in the presence of NMDAr blockers (black) and NMDAr blockers + apamin (red) (n = 21 and 22 spines respectively). Scale bar: 6 pA (top), 0.45 mV (middle) and 6% ΔG/R (bottom), and 25 ms for panels (a) and (b). The shaded region shows the mean ± SEM for each average trace.

c, Comparison of the Ca^{2+} transient amplitude and rise time each condition. * indicates $p < 0.05$ compared to control.

Figure 7

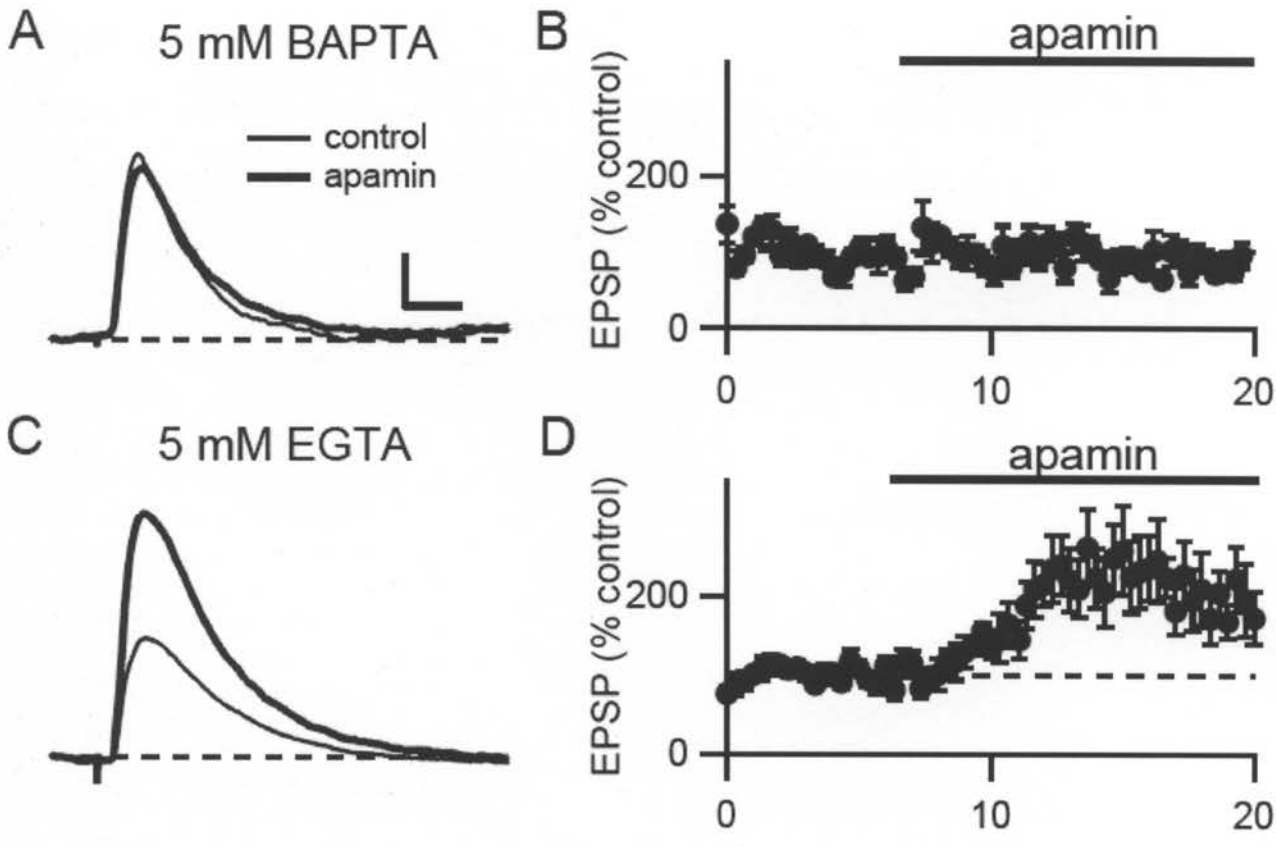

A
5 mM BAPTA
control
apamin
B
apamin
EPSP (% control)
200
0
0
10
20
C
5 mM EGTA
D
apamin
EPSP (% control)
200
0
0
10
20

Figure 7 Effects of BAPTA and EGTA on the apamin-induced increase of the EPSP

a and c, Synaptically-evoked EPSPs recorded in control conditions (thin traces) or in the presence of apamin (thick traces) with either 5 mM BAPTA or 5 mM EGTA in the patch pipette as indicated.

b and d, Summary plots of the effects of apamin on EPSP peaks for intracellular solutions containing 5 mM BAPTA (n = 10 cells) or 5 mM EGTA (n = 8 cells), respectively. The time of apamin application is indicated by the bars. Scale bar: 1 mV and 25 ms.

Table 1 Effects of apamin on synaptically evoked EPSPs

Condition (*n*)	EPSP (mV)		Slope (V/s)		HW (ms)	Rise time (ms)
Control	3.3 ± 0.4		0.84 ± 0.13		35 ± 4	4.5 ± 0.2
Apamin	5.6 ± 0.5	(173 ± 16%)[a]	1.14 ± 0.09	(141 ± 12%)[a]	40 ± 3	5.0 ± 0.1
Apamin + AP5 (5)	2.7 ± 0.6	(79 ± 13%)	0.70 ± 0.10	(84 ± 8%)	33 ± 5	4.9 ± 0.3
Control	3.4 ± 0.5		0.72 ± 0.11		44 ± 9	6.6 ± 1.0
dTC	7.0 ± 1.2	(216 ± 53%)[a]	0.96 ± 0.09	(146 ± 23%)[a]	47 ± 6	8.7 ± 0.9
Washout (6)	5.1 ± 0.8	(154 ± 21%)	0.90 ± 0.10	(131 ± 11%)[a]	41 ± 10	6.1 ± 0.9
Control	4.1 ± 0.3		0.96 ± 0.07		40 ± 8	4.6 ± 0.6
AP5	3.2 ± 0.3	(78 ± 5%)[a]	0.76 ± 0.05	(80 ± 4%)[a]	44 ± 8	4.8 ± 0.5
AP5 + Apamin (7)	3.1 ± 0.3	(96 ± 3%)	0.71 ± 0.06	(93 ± 5%)	48 ± 8	5.9 ± 1.1
Control (0.2 mM Mg^{2+})	1.7 ± 0.2		0.62 ± 0.07		56 ± 7	7.4 ± 1.4
Apamin (6)	4.3 ± 0.2	(266 ± 29%)[a,b]	1.00 ± 0.08	(167 ± 10%)[a]	43 ± 3	6.1 ± 1.1
Control (CNQX)	1.3 ± 0.3		0.5 ± 0.2		82 ± 22	10.8 ± 3.2
Apamin (9)	4.1 ± 1.0	(320 ± 52%)[a]	0.7 ± 0.2	(171 ± 20%)[a]	105 ± 26	16.1 ± 4.5
AP5 (5)	0.3 ± 0.1	(12 ± 18%)	nd		Nd	nd
Control (5 mM BAPTA)	2.5 ± 0.6		1.55 ± 0.61		35 ± 4	5.0 ± 0.5
Apamin (6)	2.1 ± 0.5	(89 ± 3%)	1.47 ± 0.57	(104 ± 12%)	35 ± 4	4.8 ± 0.8
Control (5 mM EGTA)	3.5 ± 0.7		0.86 ± 0.12		40 ± 5	4.4 ± 0.7
Apamin (8)	6.7 ± 0.9	(214 ± 32%)[a]	1.41 ± 0.17	(170 ± 15%)[a]	35 ± 5	4.4 ± 0.3
Control (1 mM BAPTA)	5.8 ± 0.8		1.16 ± 0.12		27 ± 8	4.6 ± 0.5
Apamin (5)	7.8 ± 0.7	(140 ± 11%)[a]	1.66 ± 0.13	(147 ± 15%)[a]	27 ± 7	4.5 ± 0.5

Properties of synaptically evoked responses. Slope is defined as the maximum rate of rise of the EPSP. Rise time is defined as the time required for the EPSP to rise from 20 to 80% of the maximum amplitude. Half-width (HW) is the width of the synaptic potential measured at 50% of the maximum amplitude. Numbers in parentheses are percentage of the control for each condition. [a]$P < 0.05$ compared with control. [b]$P < 0.05$ compared with increase in normal bath solution.

Table 2. Properties of single spine uncaging-evoked responses

Condition	uEPSC			uEPSP		
	Amp (pA)	Risetime (ms)	HW (ms)	amp (mV)	risetime (ms)	HW (ms)
Control (n=24)	-15.1±0.5 (100±3%)	1.1±0.1 (100±9%)	6.7±0.5 (100±7%)	0.92±0.09 (100±10%)	2.8±0.3 (100±11%)	30±3 (100±10%)
Apamin (n=25)	-14.7±0.5 (97±3%)	1.4±0.1 (127±13%)	8.0±0.7 (119±10%)	1.00±0.07 (109±8%)	3.4±0.3 (121±11%)	45±4* (150±13%)
CPP + MK801 (n=21)	-14.7±0.8 (97±5%)	1.3±0.1 (118±13%)	7.6±0.7 (113±10%)	1.02±0.11 (111±12%)	3.3±0.3 (118±9%)	37±3 (123±10%)
Apamin + CPP + MK801 (n=22)	-15.0±0.8 (99±5%)	1.3±0.1 (118±13%)	8.5±0.6 (126±9%)	1.08±0.10 (117±11%)	2.5±0.2 (89±7%)	30±3 (100±10%)

Risetime is defined as the time required to rise from 20 of 80% of the maximum amplitude. HW is the width of the synaptic current or potential measured at 50% of the maximum amplitude. In parenthesis are the mean and SEM in each condition expressed as a percentage of the mean value in the "control" condition. * indicates $p<0.05$ by ANOVA for comparison between the value in control and apamin conditions or between the value in CPP+MK801 and apamin+CPP+MK801 conditions.

Apamin-sensitive SK2 channels contribute to the generation of the post-tetanus afterhyperpolarization in CA1 neurons

ABSTRACT

A classically ascribed role for apamin-sensitive SK channels is that they contribute to the medium afterhyperpolarization (AHP) that follows an action potential (AP), influencing spike frequency and bursting behavior. SK channels are abundantly expressed in CA1 neurons, but here are conflicting conclusions about whether SK channels contribute to the medium component of the AHP in these cells. We examined the AHP in CA1 neurons following a sustained current injection or bursts of action potentials elicited at different frequencies using brain slices from wildtype and SK2 null mice. Our results show that apamin-sensitive SK2 channels make a clear contribution to the medium component of the AHP.

INTRODUCTION

Small conductance Ca^{2+}-activated K^+ channels are widely expressed throughout the mammalian central nervous system (Stocker and Pedarzani, 2000). SK channels are potently and very specifically blocked by the bee venom peptide apamin that provides a distinct profile for the consequences of SK channel activity (Blatz and Magleby, 1986; Sah et al., 1996; Köhler et al., 1996). In several types of neurons, such as pyramidal neurons, apamin application inhibits the medium component of the AHP and increases neuronal excitability (Abel et al., 2004). In contrast, hippocampal CA1 neurons were thought to lack apamin-sensitive channels, but more recently an apamin-sensitive current was reported and data were presented showing that apamin reduced the mAHP and increased excitability (Stocker et al., 1999; Sah and Clements, 1999). It was subsequently shown that the apamin sensitive current was abolished in SK2 null mice (Bond et al., 2004). However, a more detailed study has now refuted the earlier conclusions, showing that apamin did not affect the number of APs elicited by a given current injection nor the post-tetanus AHP, and it was proposed that SK channels do not contribute to the AHP or affect excitability. Rather, it was concluded that a combination of M-channel and h-channel activities underlies the mAHP in CA1 neurons (Gu et al., 2005).

We have examined the AHP in wildtype and SK2 null mice, using either prolonged current injections, or a varying number of action potentials elicited at different stimulation frequencies. The results show that SK2 channels modulate the interspike AHP and the post-tetanus AHP. The influence of SK channels is activity-dependent becoming more prominent at physiologically relevant firing frequencies.

RESULTS

First, we performed current-clamp recordings in which we elicited 400 ms-long bursts of action potentials. Such bursts were followed by a bi-phasic afterhyperpolarization, comprising the mAHP and a slower phase, the slow AHP (sAHP) (Fig. 1a). The mAHP was reduced upon application of the selective SK channel blocker apamin (50 nM; Fig. 1a), which did not affect the sAHP. The reduction of the mAHP was specifically due to the blockade of SK channels, as supported by the concomitant reduction of I_{mAHP}, but not of I_{sAHP}, in voltage-clamp recordings (Fig. 1b). The mAHP could be stably recorded in response to action potential bursts for up to 20 minutes (Fig. 1c), and was reduced by 40.0 ± 7.3% by apamin (Fig. 1d; n=5). This results shows that SK channels are activated by bursts of action potentials and contribute to the generation of the mAHP inCA1 pyramidal neurons.

The effect of SK channels with apamin was next examined using a series of 20 action potentials evoked at different frequencies. Figure 2a shows the effects of apamin (100nM) on the post-tetanus AHP for a representative cell stimulated at 50 Hz. In this cell, apamin reduced the post-tetanus AHP from 8.6 mV to 4.3 mV, and across 9 cells tested from 6.6 ± 0.9 mV to 3.0 ± 0.8 mV ($p < 0.01$). Evoking 20 action potentials at different frequencies (5, 10, 20, 50 Hz) showed that apamin had a significant impact on the post-tetanus AHP at all frequencies tested ($p < 0.04$, $n \geq 5$; Fig. 2b). Blocking SK channels with apamin also affected the development of the interspike AHP during he trains of action potentials. Fig. 2c shows a diary plot of the interspike AHP for a representative cell, evoked at 50 Hz. In control and apamin conditions an interspike AHP

develops early in the train of action potentials. In the absence of apamin this interspike AHP continues to grow throughout the train, while in the presence of apamin the interspike AHP remains stable after the fourth action potential. In control conditions there was a significant difference between the first and the last interspike AHPs at 20 and 50 Hz ($p < 0.02$), however there was no difference between the first and the last interspike AHPs at these frequencies after application of apamin. At 10 and 5 Hz there was no significant difference in the amplitude of the first and the last interspike AHPs wither in control or apamin conditions ($p > 0.05$; Fig. 2d).

We then examined the effects of apamin on the post-tetanus AHP following trains of different numbers of action potentials (5, 10, 20 and 50) elicited at 50 or 20 Hz. Fig. 3a shows a representative cell for 50 action potentials evoked at 50 Hz. In this cell apamin reduced the post-tetanus AHP from 8.1 to 3.2 mV and across 10 cells tested, from 6.4 ± 0.5 mV to 2.9 ± 0.4 mV. For all numbers of action potentials tested, apamin significantly reduced the post-tetanus AHP ($p < 0.05$; $n > 7$; Fig. 3b). Similar results were obtained at 20 Hz (not shown). SK2 channels are highly expressed in CA1 neurons and the apamin sensitive current is abolished in Sk2 null mice (Bond et al., 2004). When 20 action potentials were evoked at different frequencies in slices prepared from Sk2 null mice, apamin had no effect on the post-tetanus AHP ($p > 0.4$, $n = 4$; Fig. 3c,d), nor was the interspike AHP affected (not shown). To determine whether the apparent effect of apamin on the post-tetanus AHP may be due to rundown of other channels during the experiment we monitored the post-tetanus AHP following 20 action potentials evoked at 50 Hz every minute for 12 minutes. Slices were incubated in either control solutions for

the entire experiment or were treated with apamin after the fourth minute. In control conditions, the post-tetanus AHP was not different after the first or the last set of action potentials (5.4 ± 0.7 mV, first set; 5.1 ± 0.4 mV, last set; $p > 0.05$. $n = 6$), while apamin reduced the post-tetanus AHP (5.8 ± 0.2 mV, first set, 4.09 ± 0.6 mV, last set; $p = 0.02$, $n=3$). Therefore the effects of apamin on the post-tetanus AHP are not due to rundown, and reflect the block of apamin-sensitive SK channels.

M channels have been implicated in contributing to the medium AHP in CA1 neurons (Storm, 1989; Gu et al., 2005, Peters et al., 2005). Figure 4a shows the effects of the M channel blocker XE991 (10 μM) and the effects of XE991 plus apamin on the post-tetanus AHP following 50 action potentials for a representative cell stimulated at 50 Hz. In this cell, XE991 reduced the post-tetanus AHP following 50 action potentials from 5.9 to 4.5 mV, while subsequent co-application of XE991 and apamin reduced the post-tetanus AHP to 2.5 mV, and across 7 cells tested XE991 reduced the post-tetanus AHP from 6.3 ± 0.9 to 5.6 ± 1 mV ($p = 0.59$), and XE991 plus apamin reduced the post-tetanus AHP to 3.2 ± 0.7 mV ($p = 0.006$). M channels activate slowly, so we repeated the experiments using stimulations at 20 Hz. In contrast to the results obtained at 50 Hz, when XE991 was applied during 20 Hz stimulations, the post-tetanus AHP was significantly reduced for 10, 20 and 50 action potentials ($p < 0.03$), while application of XE991 plus apamin further reduced the post-tetanus AHP ($p < 0.04$, $n > 5$).

DISCUSSION

The results presented here demonstrate that apamin-sensitive SK channels are activated by bursts of action potentials and contribute to the generation of the mAHP inCA1 pyramidal neurons from both rat and mouse. The loss of an apamin-sensitive current (Bond et al., 2004) and AHP component in SK2 null mice suggests that SK2-containing channels are necessary for the SK contribution to the AHP. The contribution of SK channels to the generation of the mAHP in CA1 pyramidal neurons has been observed in some studies (Stocker et al., 1999; Oh et al., 1999; Kramar et al., 2004), but not in others (Gu et al., 2005). The latter study suggested that previous results showing an SK contribution to the mAHP might have been due to run-down of other conductances that were mistaken for apamin-sensitive SK channel activity. However, we found that the mAHP was stably recorded for prolonged periods of time in response to action potential bursts.

SK channels also contributed to the post-tetanus AHP that followed a series of elicited action potentials. The reduction of the post-tetanus AHP caused by blocking SK channels was frequency-independent; apamin reduced the post-tetanus AHP following 20 action potentials by approximately the same amount regardless of the frequency at which the action potentials were evoked. Hippocampal CA1 neurons fire over a wide range of frequencies. For example, place cells use the relationship between phase of firing relative to the theta oscillations and firing rates to encode information about their spatial environment. These aspects are dissociable and encode different sets of information

(Huxter et al., 2003). The firing rate reflects the speed of movement and other behavioral and environmental information, and may exceed 50 Hz (Huxter et al., 2003; Mehta et al., 2002). Our results show that even at firing rates consistent with theta oscillations such as 5 Hz, blocking SK channels reduced the post-tetanus AHP. Similar results were obtained at significantly faster firing rates, such as 20 and 50 Hz. Therefore, activity-dependent SK channel activation will affect the excitability of CA1 neurons across a wide spectrum of physiologically relevant firing rates.

M channels have also been implicated as contributing to the mAHP (Storm, 1989; Gu et al., 2005, Peters et al., 2005). In contrast to the effect of apamin, blocking M channels with XE991 reduced the post-tetanus AHP in a frequency-dependent manner. XE991 did not affect the post-tetanus AHP when action potentials were elicited at 50 Hz, but did reduce the post-tetanus AHP when action potentials were elicited at lower frequencies such as 20 Hz. In both cases, subsequent co-application of apamin reduced the post-tetanus AHP. It has been suggested that M channels contribute to the AHP when the resting membrane potential is relatively depolarized, such as -60 mV, but not for more negative resting potentials such as -80 mV. In our experiments that examined the contribution of M channels, the membrane potential was not clamped and was between -68 and -71 mV for 50 Hz stimulations, and between -71 and -73 mV for 20 Hz stimulations. Yet, XE991 only reduced the post-tetanus AHP at 20 Hz. Therefore the effects seem to be frequency-dependent and not due to a more positive resting membrane potential during the experiments that used 20 Hz stimulations.

Taken together with previous results, the present findings suggest that M channels, h-channels and SK channels contribute to the mAHP in CA1 neurons. The relative contribution of M- and h-channels may vary with resting potential and firing frequency, while SK channels contribute throughout.

MATERIALS AND METHODS

Slice preparation. All procedures were done in accordance with the guidelines of Oregon Health & Science University. Hippocampal slices were prepared from C57BL/6J mice from postnatal week 4-6 for electrophysiology experiments. Animals were anesthetized by intraperitoneal injection with a ketamine/xylocaine cocktail, and decapitated. The cerebral hemispheres were quickly removed and placed into cold artificial CSF and equilibrated with 95%O_2/5%CO_2. Hippocampi were removed, placed onto an agar block, and transferred into a slicing chamber containing sucrose-ACSF. Transverse hippocampal slices (350 μm) were cut with a Leica VT1000s (Leica Instruments, Nussloch, Germany) and transferred into a holding chamber containing regular ACSF (in mM): 125 NaCl, 2.5 KCl, 21.4 $NaHCO_3$, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 11.1 glucose and equilibrated with 95%O_2/5%CO_2.

Slices were incubated at 34°C for 30 min and then allowed to recover at room temperature for ≥1.5 hrs before recordings were performed.

Electrophysiology. For recordings, CA1 pyramidal cells were visualized with IR/DIC optics (Zeiss Axioskop 2FS) and a CCD camera (Sony, Tokyo, Japan). Whole-cell patch-clamp recordings were obtained from CA1 pyramidal cells using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA), digitized using an ITC-16 analog-to-digital converter (InstruTech, Port Washington, NY) and transferred to a computer using Pulse software (Heka Elektronik, Lambrecht/Pfalz, Germany).

Patch pipettes (open pipette resistance, 3.5-5.5MΩ) were filled with a solution containing (in mM): 120 potassium gluconate, 10 HEPES, 0.1 EGTA, 20 KCl, 2$MgCl_2$, 10

phosphocreatine, 2 Na_2ATP, 0.25 GTP (pH 7.3). Bursts of action potentials (APs) were recorded in whole-cell current-clamp mode. Action potentials were elicited by somatic 100 μs current injections. The input resistance was determined from a ~7 pA hyperpolarizing current injection pulse given 4800 ms after each AP burst. Recordings were made using an Axon 200A amplifier (Axon Instruments, Union City, CA) interfaced to a Macintosh G4 with an ITC-16 computer interface (Instrutech Corp, Port Washington, NY). Data was filtered at 5 kHz and collected at a sample frequency of 20 kHz using Pulse (Heka Elektronik, Lambrecht/Pfalz, Germany). All recordings used cells with a resting membrane potential <-60 mV that did not change by more than 4 mV during an experiment and a stable input resistance.

Data analysis. Data were analyzed using IGOR (WaveMetrics, Lake Oswego, OR). Paired t-tests was used to determine significance; $p < 0.05$ was considered significant.

Pharmacology. Apamin (Calbiochem, La Jolla, CA) and XE991 (Tocris Cookson, Ellisville, MO) were dissolved in distilled water.

Figure 1:

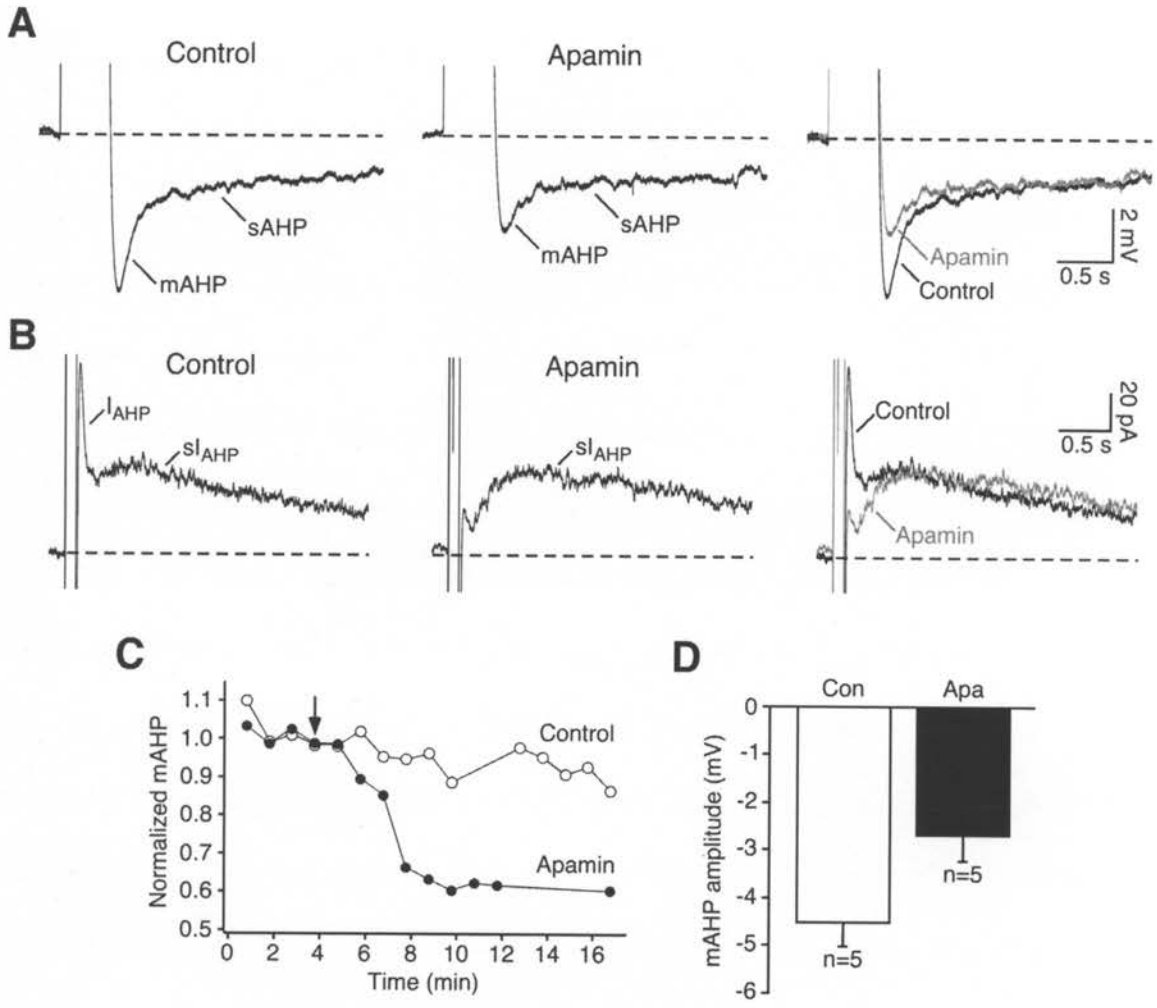

A
Control
Apamin
sAHP
mAHP
sAHP
mAHP
Apamin
Control
2 mV
0.5 s
B
Control
Apamin
I_{AHP}
sI_{AHP}
sI_{AHP}
Control
Apamin
20 pA
0.5 s
C
Normalized mAHP
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0
2
4
6
8
10
12
14
16
Time (min)
Control
Apamin
D
mAHP amplitude (mV)
0
-1
-2
-3
-4
-5
-6
Con
Apa
n=5
n=5

Figure 1:

a. Bursts of action potentials (truncated in the figure) were elicited by 400 ms long current injections (400 pA). Bursts were followed by a mAHP and a sAHP (left panel). While the mAHP was reduced upon application of 50 nM apamin, the sAHP was unaffected (middle panel). The right panel shows a superimposition of the traces before (control) and after apamin application. The dotted line corresponds to the membrane resting potential (-57 mV).

b. I_{AHP} and sI_{AHP} were elicited in voltage-clamp recordings by 100 ms long pulses to +10 mV from a holding potential of –50 mV. Apamin (50 nM) reduced I_{AHP} without affecting sI_{AHP} (middle panel). The right panel shows a superimposition of the traces before (control) and after apamin application. The traces are from the same cell as in A.

c. Time course of action of apamin (50 nM) on the mAHP. The mAHP was generated by action potential bursts as shown in a, elicited every 20 s. Each symbol corresponds to the average normalized amplitude of three mAHP elicited each minute. The open circles correspond to the mAHP in a long control recording, where no apamin was applied. The closed circles illustrate the time course of action of apamin in the same cell as in a. The time at which apamin was applied is indicated by an arrow. Similar time courses were observed in 5 cells.

d. Bar diagram summarizing the effect of apamin (50 nM) on the amplitude of the mAHP in 5 cells. The mean amplitude of the mAHP following action potential bursts triggered by 400ms long current injections (400 pA) was 4.5±0.5 mV before, and 2.7 ± 0.5 mV after the application of apamin.

Figure 2:

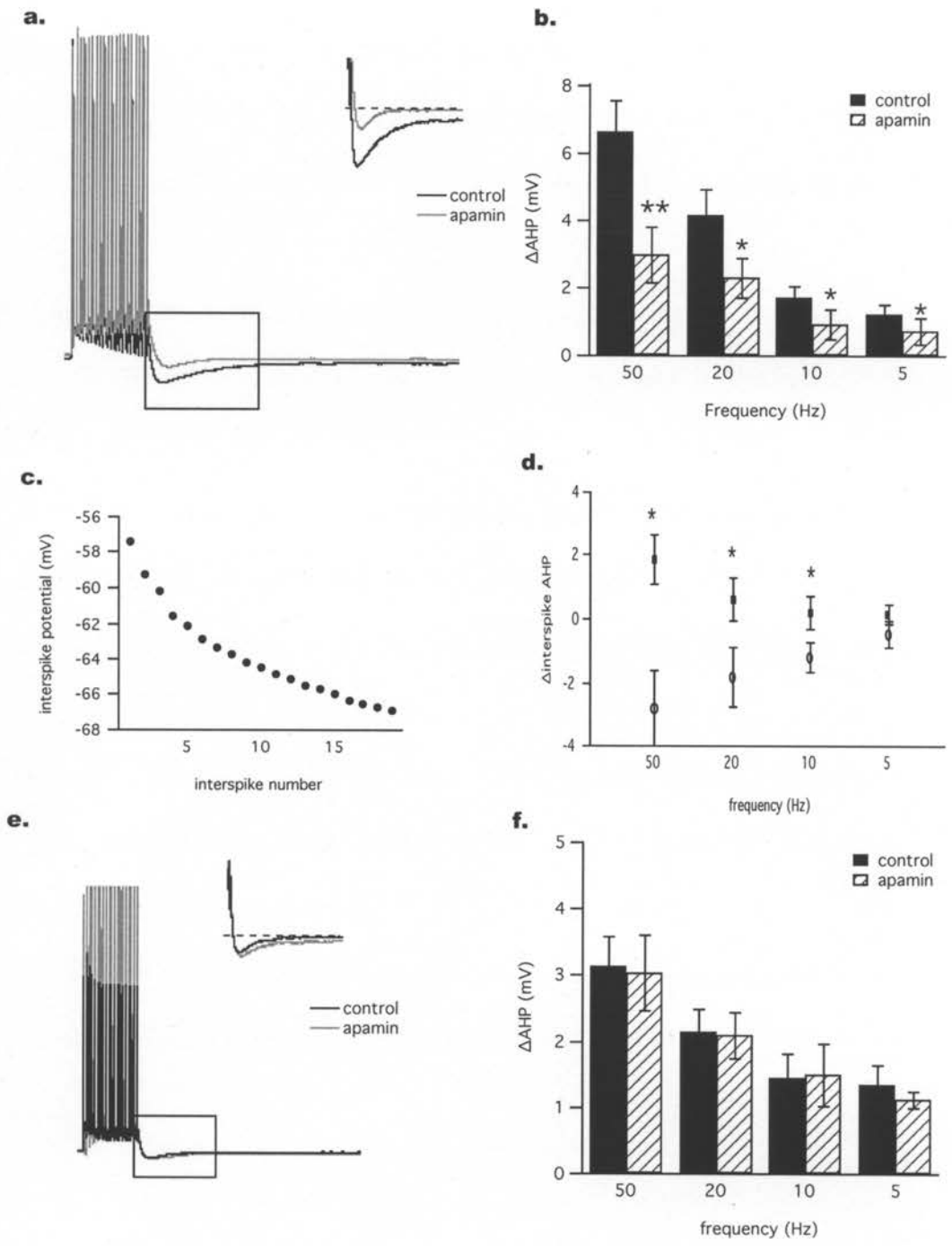

a.
control
apamin
b.
control
apamin
ΔAHP (mV)
Frequency (Hz)
50
20
10
5
c.
interspike potential (mV)
interspike number
d.
Δinterspike AHP
frequency (Hz)
e.
control
apamin
f.
control
apamin
ΔAHP (mV)
frequency (Hz)

Figure 2: Effect of apamin on the post-tetanus AHP in wildtype and SK2-null mice, elicited by a burst of 20 action potentials at different frequencies

a. Bursts of 20 action potentials were elicited by current injection of 20 100 μs pulses in wildtype mice. The bursts were followed by an afterhyperpolarization (AHP), which was reduced upon application of 100nM apamin. Inset: Post-tetanus AHP on an expanded scale.

b. Bar diagram summarizing the effect of 100 nM apamin on the amplitude of the AHP for bursts of 20 action potentials at 50, 20, 10 and 5 Hz. ($n = 9, 7, 6$ and 5; *: $p < 0.05$, **: $p < 0.01$).

c. Effect of 100 nM apamin on the interspike-AHP: Apamin led to a gradual reduction of the amplitude of the interspike-AHP the cell shown in (a).

d. Effect of 100 nM apamin on the last interspike-AHP for bursts of 20 action potentials at 50, 20, 10 and 5 Hz. ($n = 4, 4, 4$ and 4).

e. Bursts of 20 action potentials were elicited by current injection of 20 100 μs pulses in SK2-null mice. Apamin did not have an effect on the post-tetanus AHP. Inset: Post-tetanus AHP on an expanded scale.

f. Bar diagram summarizing the effect of 100 nM apamin on the amplitude of the AHP for bursts of 20 action potentials at 50, 20, 10 and 5 Hz. ($n = 4, 4, 4$ and 4).

Figure 3:

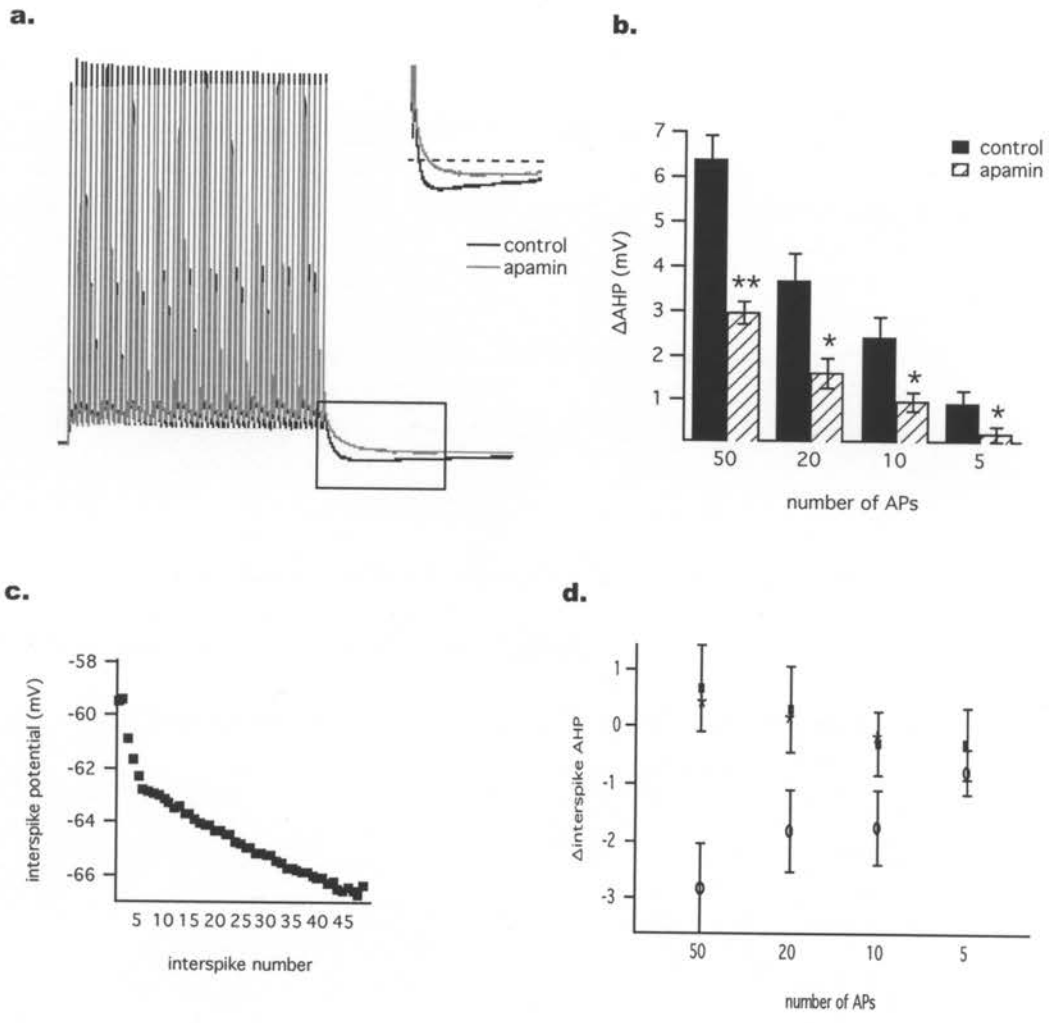

a.
control
apamin
b.
control
apamin
ΔAHP (mV)
7
6
5
4
3
2
1
**
*
*
*
50
20
10
5
number of APs
c.
interspike potential (mV)
-58
-60
-62
-64
-66
5 10 15 20 25 30 35 40 45
interspike number
d.
Δinterspike AHP
1
0
-1
-2
-3
50
20
10
5
number of APs

Figure 3: Effect of apamin on the post-tetanus AHP, elicited by a burst of action potentials at 50 Hz

a. Bursts of 50, 20, 10 and 5 action potentials at 50 Hz were elicited by current injection of 100 μs pulses. The bursts were followed by an afterhyperpolarization (AHP), which was reduced upon application of 100 nM apamin. Inset: Post-tetanus AHP on an expanded scale.

b. Bar diagram summarizing the effect of 100 nM apamin on the amplitude of the AHP for bursts of 50, 20, 10 and 5 action potentials at 50 Hz. ($n = 10, 9, 8$ and 8; *: $p < 0.05$, **: $p < 0.01$).

c. Effect of 100 nM apamin on the interspike-AHP: Apamin led to a gradual reduction of the amplitude of the interspike-AHP the cell shown in (a).

d. Effect of 100 nM apamin on the last interspike-AHP for bursts of 50, 20, 10 and 5 action potentials at 50 Hz ($n = 10, 9, 8$ and 8; *: $p < 0.05$).

Figure 4:

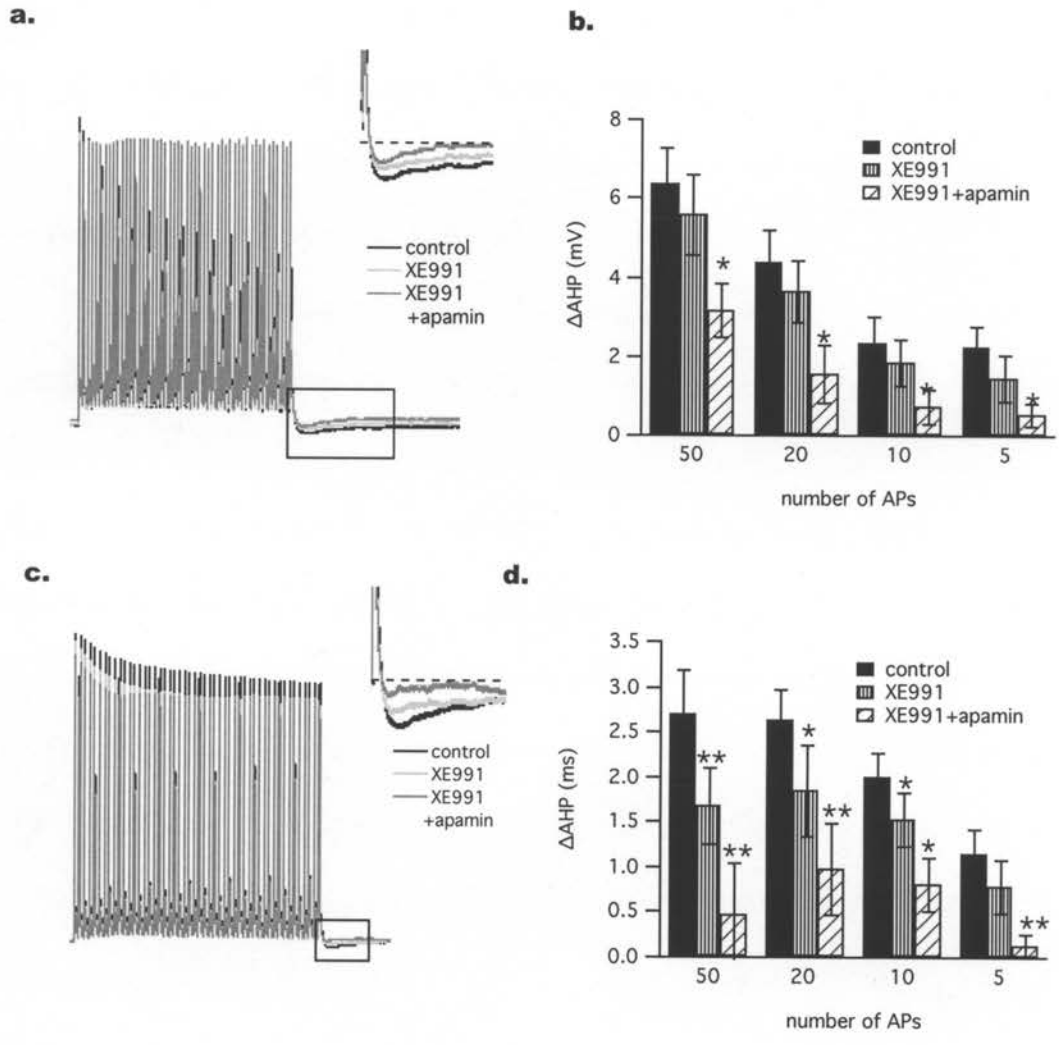

a.
control
XE991
XE991
+apamin
b.
control
XE991
XE991+apamin
ΔAHP (mV)
8
6
4
2
0
50
20
10
5
number of APs
c.
control
XE991
XE991
+apamin
d.
control
XE991
XE991+apamin
ΔAHP (ms)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
50
20
10
5
number of APs

Figure 4: Effect of XE991 and apamin on the post-tetanus AHP, elicited by a burst of action potentials at 50 Hz and 20 Hz

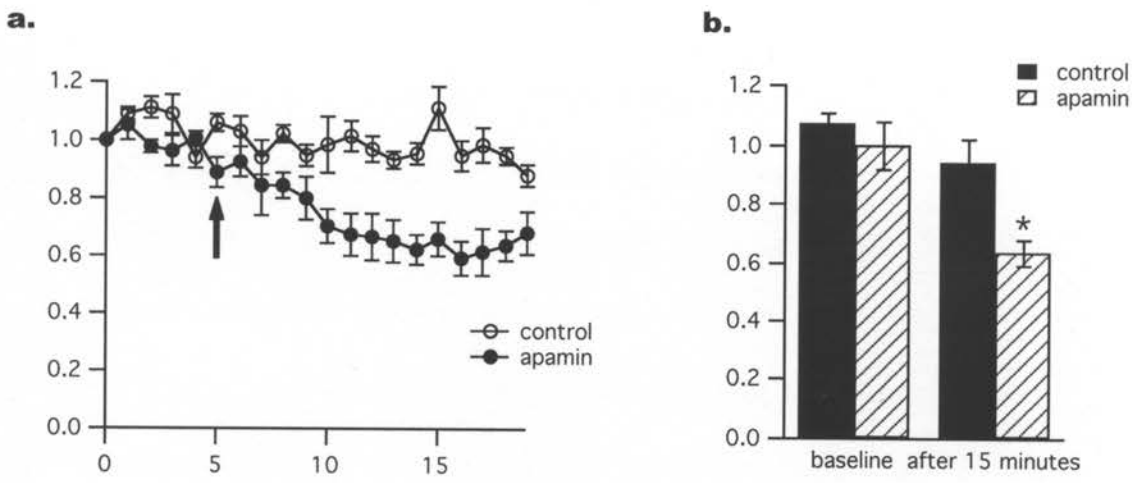
a. Bursts of 20 action potentials at 50 Hz were elicited by current injection of 100 μs pulses. The M-channel blocker XE991 (10 μM) did not reduce the amplitude of the AHP, whereas subsequent application of 100 nM apamin led to a reduction of AHP amplitude. Inset: Post-tetanus AHP on an expanded scale.

b. Bar diagram summarizing the effect of 10 μM XE991 and 100 nM apamin on the amplitude of the AHP for bursts of 50, 20, 10 and 5 action potentials at 50 Hz ($n = 7, 7, 6$, and 4; *: $p < 0.05$).

c. Bursts of 20 action potentials at 20 Hz were elicited by current injection of 100μs pulses. The M-channel blocker XE991 (10μM) did not reduce the amplitude of the AHP, whereas subsequent application of 100 nM apamin led to a reduction of AHP amplitude. Inset: Post-tetanus AHP on an expanded scale.

d. Bar diagram summarizing the effect of 10 μM XE991 and 100 nM apamin on the amplitude of the AHP for bursts of 50, 20, 10 and 5 action potentials at 20 Hz. ($n = 9, 11, 8$ and 9; *: $p < 0.05$, **: $p < 0.01$).

Supplemental figure:



IV. DISCUSSION AND FUTURE DIRECTIONS

The hippocampus is an essential neurobiological substrate for certain forms of learning and memory. The work presented here demonstrates that SK channels play an important role in regulating hippocampal function. Specifically, the data from both studies indicate that apamin-sensitive SK channels represent a neural mechanism that is capable of regulating hippocampal-dependent memory. The distinct distribution of SK channel subunits, despite their functional similarities in heterologous systems, argues in favour of mechanisms of regulation that confer distinct and specific functional profiles to native SK channels. The availability of highly specific pharmacological tools together with the detailed mapping of SK channel distribution have opened the way to studies addressing the molecular identity of native SK channels in different brain regions and to correlate Ca^{2+}-activated K^+ currents with their molecular counterparts.

Neuronal potassium channels, which were initially thought to be responsible for controlling cellular firing properties, have increasingly been shown to have key roles in dendritic integration by shunting local dendritic excitation. In this framework, in the first part of this thesis, a variety of pharmacological manipulations were used to investigate how SK channels contribute to shaping the hippocampal postsynaptic response of CA1 neurons. In the second part of this thesis, pharmacology and different recording situations were used to assess the role of SK channels in the generation of the afterhyperpolarization following bursts of action potentials.

Taken together, these studies support the current notion that functionally distinct SK2 channels in the soma, dendrites and dendritic spines are embedded in distinct

microdomain signaling complexes, where they are coupled to different Ca^{2+} sources. The different subcellular localizations and microdomain organizations allow each SK subtype to contribute to different aspects of neuronal function.

SK channel function in different systems

Coupling of Ca^{2+} sources to Ca^{2+}-activated K^+ channels

Intracellular Ca^{2+} is a versatile second messenger in neurons. Synaptic activity induces Ca^{2+} entry in hippocampal pyramidal cells; until buffering mechanisms restore resting Ca^{2+} levels, free cytoplasmic Ca^{2+} regulates critical cellular functions, including neurotransmitter release, gene transcription and channel modulation. Furthermore, varieties of neural events such as long-term potentiation (Berridge, 1998; Lynch et al. 1983), long-term depression (Rose et al., 2001; Sakurai, 1990), electroencephalographic (EEG) rhythm generation (McCormick et al., 2001), and neural cell death (Choi, 1994) are all dependent on intracellular Ca^{2+} increases. Yet, once intracellular Ca^{2+} is increased, one of such Ca^{2+}-dependent events, but not the others, is specifically triggered. How can versatility and specificity co-exist in neural Ca^{2+} signaling? Much of this versatility arises through the diverse mechanisms by which Ca^{2+} signals are generated and transmitted to act over very different time and distance scales. For example, Ca^{2+} can act in a very fast and highly localized manner, as in the triggering of neurotransmitter release within microseconds of Ca^{2+} entry through voltage-gated channels closely apposed to active release zones (Neher, 1998); or it can evoke slower responses involving global Ca^{2+}

elevations throughout the cell, as in the generation of Ca^{2+}-dependent Cl^- currents by Ca^{2+} waves that take many seconds to sweep across *Xenopus* oocytes (Parker and Yao, 1994). An emerging view related to this question of specificity is that Ca^{2+}-channels and Ca^{2+}-activated channels form functional complexes, and each complex may be dedicated to one particular function. In this picture, Ca^{2+} elevations are largely permissive, with localized interactions in signaling complexes providing specificity.

For example, in outside-out membrane patches from rat olfactory bulb granule cells a specific linkage of *N*-methyl-D-aspartate receptors (NMDA receptors) and large-conductance Ca^{2+}-dependent K^+ channels (BK channels) enables a synaptically triggered, extra-synaptic inhibition (Isaacson and Murphy, 2001). The resulting inhibition is long lasting, which may be critical to the operation of the dynamic circuitry of the olfactory bulb. Similarly, NMDA application evoked a Ca^{2+}-dependent K^+ current in cultured hippocampal neurons (Zorumski et al., 1989). Furthermore, Ca^{2+} influx through NMDA receptors is known as a particularly suitable activator of ryanodine receptors in hippocampus pyramidal cell spines, suggesting a functional coupling between NMDA receptors and ryanodine receptors (Emptage et al., 1999). Lastly, an intimate link is proposed between voltage-dependent Ca^{2+}-channels and Ca^{2+}-dependent cationic channels, which contributes to generation of spike afterdepolarization (Partridge et al., 1999).

Ca^{2+} entry during repetitive firing serves as a feedback regulator of firing rate, activating Ca^{2+}-dependent K^+ channels that produce afterhyperpolarizations and spike frequency adaptation. This feedback increases the dynamic firing range and modifies

information content of spike output, based on recent firing behavior (Wang et al., 1998). SK channels, which are activated voltage-independently and deactivate with a slow time course after single or bursts of action potentials (Hirschberg et al., 1998), play the principal role in evoking the medium AHP current in hippocampal CA1 neurons.

Coupling of different Ca^{2+} sources and Ca^{2+}-activated K^+-channels may differ between cell types and intracellular compartments. Segregration of coupling between channel types has been proposed to result from microdomains of submembrane Ca^{2+} (e.g. Marrion et al., 1998). In different cell types (e.g. dopamine neurons of in the ventral midbrain, cerebellar Purkinje neurons, outer hair cells of the cochlea, to name a few), SK channels have been shown to be differently distributed and embedded in distinct microdomains.

SK channels and their microdomains in different systems

a. SK channels in dopaminergic neurons in the ventral midbrain

Dopaminergic neurons in the ventral midbrain, i.e., the ventral tegmental area and the substantia nigra pars compacta, are a key component of the endogenous reward circuit. They are transiently activated by the detection, perception, and expectation of rewards, suggesting that they are under the control of highly processed inputs from the cerebral cortex and other brain regions (Schultz et al., 1993). Moreover, a subset of these neurons degenerates in Parkinson's disease and dysfunction of this neurotransmitter system may be implicated in the pathophysiology of schizophrenia and drug abuse (Grace, 1991).

Dopamine neurons of the adult exhibit intrinsic slow regular spiking, in which the pacemaker cycle is initiated by a broad action potential that is followed by a prominent afterhyperpolarization. Rebound from the afterhyperpolarization leads to another slow depolarization and a subsequent broad action potential, thus completing the pacemaker cycle. It has been demonstrated that the influx of Ca^{2+} through voltage-gated T-type Ca^{2+} channels triggered by action potentials leads to the activation of SK channels, resulting in the generation of large afterhyperpolarizations that dominate the interspike interval of pacemaker firing (Wolfart and Roeper, 2002). Blockade of SK channels with apamin revealed their central role in setting the regular spiking activity, resulting in less regular firing, as demonstrated in an increase in the coefficient of variation of the interspike interval.

In dopamine neurons of young animals, however, increases in intracellular Ca^{2+} followed by activation of SK channels have a different outcome. Cui et al. (2004) showed that spontaneous miniature outward currents are initially triggered by Ca^{2+} influx through T-type Ca^{2+} channels, which by itself is not sufficient to activate SK channels but which can activate ryanodine receptors in close apposition to the plasma membrane and induce Ca^{2+}-induced Ca^{2+}-release. The Ca^{2+} signal amplified by Ca^{2+}-induced Ca^{2+}-release is now sufficient to activate nearby SK channels, which - in contrast to the adult - causes irregular firing in the neonatal dopamine neurons. Blocking of SK channels with apamin resulted in a significant decrease in the coefficient of variation of the interspike interval. Although it is not yet known what causes the developmental switch in the effects of SK channel activation, the irregular firing mechanism might have an essential role in the development of the circuitry of the dopamine system.

Furthermore, repetitive stimulation of glutamatergic inputs evokes a slow IPSP via activation of metabotropic glutamate receptors (mGluRs) in dopamine neurons (Fiorillo and Williams, 1998). Release of Ca^{2+} from intracellular stores mediates this mGluR-induced hyperpolarization, as this rise in $[Ca^{2+}]_i$ activates SK channels on the plasma membrane. The prolonged hyperpolarization (~1 sec) results in a prolonged pause of neuronal firing, thus having a significant impact on the firing pattern of dopamine neurons. Immunohistochemistry and pharmacology identified SK3 channels as the predominant mediators of the SK effects in these cells.

b. SK channels in cochlear hair cells

Auditory outer hair cells receive synaptic input from the superior olive that controls cochlear afferent activity by inhibiting the fast voltage-dependent amplification mechanism provided by these sensory cells. Different from typical inhibitory synapses, synaptic inhibition in auditory outer hair cells is thought to be achieved by activation of neuronal nicotinic acetylcholine receptors (nACh receptor) that usually mediate excitatory postsynaptic responses. The nACh receptor in auditory outer hair cells contains the α9 subunit and supplies the postsynaptic cell with Ca^{2+} (Yuhas et al., 1999) that initiates an inhibitory hyperpolarization by opening SK channels, thus controlling fast inhibitory synaptic transmission. By monitoring the coupling between the nACh receptor and SK channels in response to stimulation of the presynapse in isolated organs of Corti, Oliver et al. (2000) demonstrate fast coupling between an excitatory ionotropic neurotransmitter receptor and an inhibitory ion channel via rapid, localized changes in subsynaptic Ca^{2+} levels. Recordings of the nACh receptor current confirmed that the

receptor behaves as a rapidly gating Ca^{2+} source that activates within $\leq$ 1 ms and deactivates with a time constant of less than 10 ms. In conclusion, tight colocalization of ACh receptors and SK2 channels in a Ca^{2+} microdomain together with the rapid gating of the Ca^{2+} source generates fast IPSCs, the time course of which is governed by the Ca^{2+} gating of the SK channel. The ACh receptor and SK channel are so closely positioned at the postsynaptic site (~10nm), that the intrinsic gating of SK2 channels rather than the rate of change determines the kinetics of the inhibitory response for intracellular Ca^{2+}.

This example, together with the slow inhibitory potentials that are triggered by glutamate through metabotropic glutamate receptors and SK channel activation in midbrain neurons (see above), indicates that the coupling between ionotropic or metabotropic receptors (leading to an increase in the intracellular Ca^{2+} concentration) with SK channels might be a general mechanism for the generation of fast or slow inhibitory synaptic transmission that is mediated by otherwise excitatory neurotransmitters.

c. SK channels in Purkinje neurons of the cerebellum

Cerebellar Purkinje neurons provide the sole output of the cerebellar cortex and play a crucial role in motor coordination and maintenance of balance. They are spontaneously active, and it is thought that they encode timing signals in the rate and pattern of their activity (Ito, 1984). The activity of a Purkinje neuron is controlled both by synaptic input and by intrinsic conductances that cause it to fire spontaneously (Womack and Khodakhah, 2002). Conductances best suited for ensuring the precision of intrinsic pacemaking are those whose activity is time-locked with an action potential.

Somatic and dendritic SK channels differently regulate the output of Purkinje neurons. The precision of pacemaking in Purkinje cells is maintained mainly by SK- and BK channels (Womack et al., 2003, 2004), which are activated by the Ca^{2+} influx associated with each action potential (Cingolani et al., 2002; Womack et al., 2003, 2004). In Purkinje cells, SK channels are exclusively activated by P/Q-type voltage-gated Ca^{2+} channels (Womack et al., 2004). This selective coupling is maintained even when Ca^{2+} influx through voltage-gated channels is increased by increasing the extracellular Ca^{2+} concentration. Womack and Khodakhah (2003) evaluated the role of apamin-sensitive SK channels in the pattern and rate of activity of Purkinje neurons. The authors found that SK channels regulate the firing of both young and adult Purkinje neurons and that somatic and dendritic SK channels play different roles in this regulation. Using single-cell extracellular spike recordings from Purkinje cells in cerebellar slices, the authors show that apamin-sensitive SK channels play a significant role in regulating the spontaneous firing of Purkinje cells, efficiently preventing the cells from firing at extremely high rates. When SK channels were blocked with bath-applied apamin, cells fired spontaneously at rates as high as 500 spikes per second. In addition, apamin was perfused locally onto the dendrites of mouse Purkinje neurons to assess the contribution of dendritic SK channels to spontaneous firing. The authors found that dendritic SK channels control primarily the extent to which dendrites contribute to the firing rate of Purkinje cells. The presence of SK channels in the dendrites allows not only for modulation of the spontaneous activity of the cell but also for control of the efficiency by which dendritic inputs are relayed to the soma, i.e. governing the efficacy of dendrosomatic electrical coupling.

Thus, in Purkinje neurons, somatic SK channels efficiently prevent the cell from firing at excessively high rates. In contrast, dendritic SK channels in Purkinje neurons modulate the efficiency of the electrical coupling of the dendrites to the soma by making the dendrites more leaky to potassium ions when internal Ca^{2+} is elevated, such as when the cell receives an input from its attendant climbing fiber. Under these conditions, the coupling efficiency would be inversely proportional to the rate of activity of the cell, decreasing the impact of synaptic inputs at higher frequencies. It is likely that block of SK channels in the proximal dendrites can affect activity of the cell by tightening the dendritic membrane and reducing dendritic leak and/or by allowing the dendrites to contribute a larger net inward current to the soma.

d. SK channels in dissociated hippocampal CA1 neurons

In cultured hippocampal neurons, it was demonstrated that Ca^{2+}-activated K^+ channels are selectively activated by co-localized Ca^{2+} channels. Marrion and Tavalin (1998) studied channel activity in cell-attached patches from acutely isolated hippocampal neurons and reported a unique specificity of coupling. L-type Ca^{2+}-channels activate SK channels only, without activating BK channels present in the same patch. The delay between the opening of the L-type Ca^{2+}-channels and SK channels indicate that these channels are 50 - 150 nm apart. In contrast, in patches from the same cell N-type Ca^{2+} channels activate BK channels only, with opening of the two channels being nearly coincident, indicating that N-type Ca^{2+}- and BK channels are very close. These results, which show the precise co-localization of CA1 somatic SK channels and L-type Ca^{2+}-channels within 100 nm, reflect the profound consequences that the precise placement of

SK channels relative to their Ca^{2+} sources on a microdomain scale will have for the kinetics and amplitude of the SK currents: for somatic SK2 channels that are gated by voltage-gated Ca^{2+}-channels, this will affect spike frequency via the mAHP, for spine SK2 channels that are gated via Ca^{2+} influx through NMDA receptors this will modulate coincidence detection and synaptic plasticity.

e. SK channels in distal dendrites of CA1 hippocampal neurons

Distal terminal apical dendrites and oblique dendrites contain the vast majority of excitatory synapses in CA1 pyramidal cells (Bannister and Larkman, 1995). These dendrites respond to strong activation with a plateau potential that is mediated by voltage-dependent Ca^{2+} channels (Wei et al., 2001). These potentials have amplitudes of 8 - 10 mV at the cell body and are generated solely in the individual dendritic branch that has been stimulated, as indicated by the spatial confinement of Ca^{2+} influx. This behavior allows each dendritic compartment to respond to strong inputs in a highly nonlinear manner and act as a quasi-independent unit of integration. These local plateau potentials are likely to be a common feature of pyramidal cell dendrites, as similar potentials have been observed in basal dendrites of pyramidal cells in acutely prepared hippocampal (Ariav et al., 2003) and cortical brain slices (Schiller et al. 2000).

In distal dendrites of CA1 neurons of the hippocampus a collaborative, functional relationship between Kv4.2 and SK channels has recently been described (Cai et al., 2004). These two potassium channel types act together to shape the Ca^{2+} plateau potentials in terminal apical or oblique dendrites of CA1 neurons. Kv4.2 channels limit the spread of the Ca^{2+} signal to a single branch, while SK channels, activated by Ca^{2+}

influx through voltage-gated Ca^{2+} channels, determine the time course by being responsible for the repolarization of the dendritic plateau potential.

Work from this thesis shows that Ca^{2+} entry that occurs through NMDA receptors after subthreshold synaptic activation will activate dendritic spine SK channels. The SK channels that are activated in this way most probably belong to a population separate from those that are activated by Ca^{2+} entry during an action potential. These findings can be explained by NMDA receptors and voltage-gated Ca^{2+}-channels having separate locations in the cell membrane, and local SK channels being activated in these respective cases.

SK channels and their role in plasticity

Synaptic plasticity

Long-lasting, activity-dependent changes in synaptic strength at excitatory synapses are thought to be critical for virtually all forms of experience-dependent plasticity, including learning and memory. Among the most widely studied and accepted models of synaptic plasticity in the mammalian brain is long-term depression (LTD) and long-term potentiation (LTP) that are generated at excitatory synapses on hippocampal CA1 pyramidal cells. These synaptic phenomena share the characteristic that they are triggered by a rise in postsynaptic Ca^{2+} concentration due to activation of NMDA receptors (Malenka, 1994). Presumably different properties of the postsynaptic Ca^{2+} signal, primarily its magnitude and perhaps its time course, activate different postsynaptic

signaling cascades that lead to either LTP or LTD (Malenka and Nicoll, 1993). As a consequence, mechanisms that alter postsynaptic Ca^{2+} underlie many forms of experience-dependent plasticity, including learning and memory. These activity-dependent long-term changes in synaptic strength are regulated by a variety of molecular substrates, including SK channels, which have been shown to regulate synaptic plasticity in CA1 pyramidal neurons (Behnisch et al., 1998; Stackman et al., 2002).

In pyramidal neurons, the sources of spine Ca^{2+} transients evoked by synaptic stimulation are controversial. While most studies suggest that Ca^{2+} influx through NMDA receptors accounts for most of synaptic spine Ca^{2+} (Yuste and Denk, 1995; Schiller et al., 1998; Kovalchuk et al., 2000; Köster and Sakmann, 1998), other results point towards Ca^{2+}-induced Ca^{2+}-release (Emptage et al., 1999) or voltage-gated Ca^{2+} channels (Schiller et al., 1998). Because less than half of CA1 spines in vivo contain smooth endoplasmatic reticulum – the organelle responsible for Ca^{2+}-induced Ca^{2+} release – the discrepancies must, at least in part, arise because of differences in preparation (cultured versus acute brain slices). Differences in recording techniques (intracellular versus whole-cell) may also contribute. The strongest case for NMDA receptors has been made by Kovalchuk et al. (2000), who showed that blockade of voltage-gated Ca^{2+} channels or Ca^{2+}-induced Ca^{2+} release does not reduce synaptic Ca^{2+} transients in brain slices. In addition, the amplitudes of synaptic Ca^{2+} accumulations obeyed the voltage dependence of NMDA receptors. In addition, the signals are blocked by the NMDA receptor antagonist APV. Although the Ca^{2+} signals can be enhanced by depolarization that relieves the Mg^{2+} block of the NMDA receptor, the signals do not require significant depolarization; they occur even when the EPSP amplitude is only a few millivolts and they are only slightly reduced

when the EPSP is blocked with CNQX. It thus appears likely that the signals occur because the NMDA receptors are in fact not completely blocked by Mg^{2+} at resting potential (Kovalchuk et al., 2000). This is also apparent by the fact that current through the NMDA receptor can be detected at resting potential (Wollmuth et al., 1998; Sabatini et al., 2001, 2002). Thus, when the glutamate from an individual vesicle is released, it partially activates NMDA receptors and thereby generates a significant Ca^{2+} signal in the postsynaptic spine. Furthermore, this Ca^{2+} influx through NMDA receptors contributes at least 75% of the postsynaptic Ca^{2+} transient that underlies changes in synaptic strength (Nevian and Sakmann, 2004).

NMDA receptors mediate changes in synaptic plasticity, due to Ca^{2+} influx through the receptor. However, increasing evidence indicates that they can also mediate more direct, short-term effects on neuronal excitation. One of the more striking examples of this phenomenon was shown recently in the olfactory bulb, at dendrodendritic synapses between excitatory mitral cells and inhibitory granule cells. NMDA receptors on granule cell dendritic spines can mediate not only a direct depolarization of granule cells, due to the influx of cations, but Ca^{2+} influx through the receptor also can trigger dendritic release of GABA (Chen et al., 2001). Within the hippocampus, NMDA receptors can mediate short-term inhibitory effects (Nicoll and Alger, 1981; Zorumski et al., 1989). Pressure-application of NMDA to cultured hippocampal neurons produced responses consisting of an initial inward cationic current followed by a slowly developing outward current carried by potassium ions. The mechanism underlying this inhibition, as well as whether such responses can be driven by synaptically released glutamate, however, has remained unclear.

SK channels and the negative feedback loop

The results from the first part of this thesis show that in hippocampal dendrites of CA1 pyramidal neurons NMDA receptors are negatively regulated by SK channels and that this mechanism explains the observed effects of apamin on hippocampal synaptic plasticity and learning and memory (Stackman et al., 2002). Activation of NMDA receptors – and the resulting Ca^{2+} influx - activates a negative feedback loop through SK channels, located within 25 – 50 nm of NMDA receptors in the spine, that depresses the synaptic potential and turns off the NMDA receptor response. During an EPSP Ca^{2+} opens SK channels, which then provide a local shunting current to reduce the EPSP and promote the reinstatement of the Mg^{2+}-dependent block of NMDA receptors and hence affect spatial and temporal integration of synaptic activity. Consistent with this, blocking SK channels enhances NMDA receptor-dependent Ca^{2+} transients in dendritic spines. The rise in Ca^{2+} in the spine head after subthreshold NMDA receptor activation has been estimated to reach at least 0.7 μM, a concentration sufficient to activate SK channels (EC_{50} = 0.3 μM). Our results indicate that this rise in postsynaptic Ca^{2+} is sufficient to activate SK channels, leading to shunting of the resulting synaptic potential.

These effects are postsynaptic, as there is no change in presynaptic transmitter release probability in the presence of apamin, and the effects of apamin are blocked by buffering postsynaptic Ca^{2+}. The fast Ca^{2+} chelator BAPTA prevented NMDA receptor-mediated Ca^{2+}-influx from activating SK channels, while the slow buffer EGTA was without effect, indicating that Ca^{2+} influx through NMDA receptors activates SK channels within a small spatial domain. These studies were performed in the presence of picrotoxin, eliminating a role for an effect via GABAergic interneurons.

The results from the first part of this thesis also demonstrated that in cultured CA1 neurons, overexpressed SK2 channels, with an external epitope-tag, were abundant in dendritic spines. Although this experiment does not reflect endogenous expression patterns, it further supports the hypothesis that SK2 channels are present in CA1 dendritic spines (and likely within proximity to NMDA receptors).

These results are consistent with reports from as early as 1989 (Zorumski et al., 1989), in which it was observed that an increase in intracellular Ca^{2+} concentration resulting from the influx of Ca^{2+} through NMDA receptors activates a Ca^{2+}-activated K^+ current in hippocampal neurons, thus providing evidence that NMDA receptors and SK channels are colocalized in these neurons (Zorumski et al., 1989; Shah and Haylett, 2002). Furthermore, our results suggest that there must be a tight coupling between NMDA receptors and SK channels such that SK channel activation tracks that of the NMDA receptor channels. This finding is similar to that seen in auditory hair cells, where SK channels are activated by Ca^{2+} influx through α9-containing nicotinic acetylcholine receptors and contribute to an IPSP (Oliver et al., 2000), and is also similar to findings in dissociated hippocampal neurons, where L-type Ca^{2+} channels activate SK channels only, without activating BK channels present in the same patch (Marrion et al., 1998).

The molecular mechanisms, which control synaptic strength, are now being elucidated at a breathtaking pace. Since NMDA receptor-mediated Ca^{2+} is critical to the processes underlying the induction of synaptic plasticity, this feedback loop most probably explains the effects of apamin on lowering the threshold for induction of LTP (Stackman et al., 2002). Consistent with this model, SK2 overexpression might be expected to increase the SK component of the EPSP. Indeed, application of apamin to

hippocampal brain slices resulted in a significantly larger increase in the NMDA receptor-mediated component of the EPSP in SK2-overexpressing than in control mice (Hammond et al., 2006). Therefore, overexpressed SK2 channels are likely to be coupled to NMDA receptors located within the dendritic spines of CA1 neurons.

It has been shown previously that different cell types employ specific subsets of Ca^{2+} sources to dictate when, where and how fast the SK channel influence on the membrane potential will be exerted (for review see: Bond et al., 2005). The data presented here support the hypothesis, that SK channels influence NMDA receptor activation through their membrane repolarization properties, which impacts the synaptically activated EPSP in the hippocampus. In the CA1 region of the hippocampus, both LTP and LTD require NMDA receptor activation. Any inhibition of SK2 channels, e.g. via CK2 phosphorylation (Bildl et al., 2004), will lead to greater membrane depolarization and an increased probability of cell firing during high-frequency excitatory input than during comparable low-frequency input. The much larger depolarization that is achieved after block of SK2 channels will allow greater Ca^{2+} influx through NMDA receptors, which contributes to the observed effect of a shift in LTP and LTD induction thresholds by apamin (Behnisch and Reymann, 1998; Stackman et al., 2002). Moreover, the shunting exerted by SK2 channels can act as a protective mechanism by reducing excitability and preventing the accumulation of intracellular Ca^{2+} to toxic levels when intense synaptic activation occurs. Therefore, SK channel modulation of NMDA receptor activity may be an important mechanism in the regulation of hippocampal synaptic plasticity – membrane repolarization (resulting from SK2 channel activation) may serve

as a physiological switch by which SK2 channels modulate the activation of synaptic NMDA receptors.

The intricate relationship between SK channels and NMDA receptors is not restricted to hippocampal neurons. Recently, Faber et al. (2005) showed in glutamatergic synapses of the lateral amygdala that Ca^{2+} influx via activated NMDA receptors also activates postsynaptic SK channels, and that activation of these SK channels depresses the synaptic potential. Analogous to the findings from the report of Stackman et al. (2002), they also demonstrate that blockade of SK channels increases LTP of cortical inputs to lateral amygdala pyramidal neurons; thus, activation of SK channels regulates synaptic transmission and synaptic plasticity in the amygdala.

Implications

Potassium channels have many regulatory mechanisms. All of the putative functions of the dendritic potassium channels could be specifically regulated in different parts of the dendritic tree. For example, trains of EPSPs or IPSPs, second messengers, modulatory neurotransmitters and changes in extracellular ions or in redox states of the cell could, in principle, modify the activation or inactivation kinetics of potassium channels and alter the delicate balance of active conductances in the dendrites. Now, more than ever, a detailed knowledge of the nuts and bolts of dendritic processing seems essential for a proper understanding of neuronal plasticity and function.

Most excitatory synapses exhibit a rich repertoire of plasticity modes that act over timescales ranging from milliseconds to weeks, and that have spatial dimensions ranging from individual synapses to all afferent synapses onto a neuron. Hebbian forms of

plasticity alter the strength of specific synapses that exhibit coincident activity. Typically, correlated presynaptic and postsynaptic firing or high-frequency stimulation results in long-term potentiation (LTP) of synaptic strength, whereas low-frequency stimulation or uncorrelated firing yields long-term depression (LTD). Such fast, durable and selective modifications of synaptic strength are necessary for wiring the brain during development and for encoding information in response to experience (Katz and Shatz, 1996). Although such synaptic change is crucial for the formation, maintenance and proper functioning of neural circuits, it raises the question of how cortical neurons keep from falling silent or keep their firing rates from saturating if the average synaptic input falls too low or rises too high. Without a mechanism that allows a neuron to remain responsive to its input in this most basic sense, the neuron would be unable to transmit information or to participate effectively in the correlation-based synaptic modifications needed for the development and maintenance of neural circuits. The answer that is emerging from recent work is that as experience-dependent plasticity changes the activity patterns of the network, several forms of homeostatic plasticity are engaged that act to restore stability to network function. Experience-dependent plasticity is thus composed of two opposing but complementary forces: one that modifies neuronal circuits progressively by creating selective differences between individual elements and another that regulates circuit properties to stabilize the overall activity of the network. This is thought to reflect behavioral experience and shapes modern concepts for the physiological basis for learning and memory.

Hebbian models for activity-dependent changes in synaptic strength are positive feedback systems and it has been suggested that the imposition of homeostatic plasticity

and metaplasticity, provide a mechanism for neural circuit stability. One proposed homeostatic mechanism suggests that the synaptic modification threshold is dynamically regulated by the level of postsynaptic activity. It serves to maintain the strengths of synapses relative to each other, lowering amplitudes of small excitatory postsynaptic potentials in response to continual excitation and raising them after prolonged blockage or inhibition (Turrigiano, 1998). This effect occurs gradually over hours or days, by changing the numbers of NMDA receptors at the synapse (Watt et al., 2004). Metaplasticity, another form of negative feedback, reduces the effects of plasticity over time (Abraham, 1996). Thus, if a cell has been affected by a lot of plasticity in the past, metaplasticity makes future plasticity less effective. Since LTP and LTD rely on the influx of Ca^{2+} through NMDA channels, metaplasticity may be due to changes in NMDA receptors, for example changes in their subunits to allow the concentration of Ca^{2+} in the cell to be lowered more quickly (Pérez-Otaño and Ehlers, 2005).

Recent studies have begun to delineate the molecular and cellular basis for these adaptations. Based on the results of the first part of this thesis, SK2 channels most probably play a role in homeostatic mechanisms in response to neuronal activity as well as being clinically relevant.

a. Metaplasticity

It is increasingly apparent that the induction of synaptic plasticity is sensitive not only to the "state" imposed by co-active afferents and circulating hormones, but also to the state generated by prior patterns of pre- and postsynaptic activity, i.e. thresholds for synaptic plasticity are not static properties of synaptic connections but, instead, vary

dynamically according to the recent history of synaptic activity. This activity-dependent modulation of subsequent plasticity has been termed 'metaplasticity' (Abraham, 1996; Abraham and Bear, 1996), the 'plasticity of plasticity'. The term is used to reflect that the prior activity has induced a change in neuronal/synaptic function that persists across time, and thus falls within the general rubric of neural plasticity. Induction of metaplasticity does not by itself lead to changes in synaptic strength; metaplasticity rather modifies the ability of a synapse to undergo further changes in response to subsequent activity. Metaplasticity thus refers to a higher-order form of synaptic plasticity where prior synaptic activity leads to a persistent change in the direction or magnitude of subsequent activity-dependent plasticity, without affecting actual synaptic efficacy (Abraham and Bear, 1996). It serves a homeostatic role because it ensures that plasticity is kept within a working range (away from saturation), i.e. within a dynamic range of modifiability. With a sliding modification threshold dependent on the average firing rate, this model addresses two problems of Hebbian plasticity. It both introduces competition between synapses and also acts to normalize synaptic weights (see below: 'Homeostatic plasticity'). It has been suggested that low-level activation of NMDA receptors in the CA1 hippocampal region induces a covert synaptic change (metaplasticity) that inhibits the subsequent induction of LTP (Abraham and Bear, 1996). The inhibition of LTP in these experiments is not absolute, however, because it can be overcome by stronger tetanic stimulation. Thus, prior stimulation appears to raise the stimulation threshold for LTP, rather than block plasticity per se.

The best-studied examples of metaplasticity are those in which prior activity shifts the threshold for LTP and LTD induction (Philpot et al., 1999). For instance, during

development of the visual cortex, high levels of coordinated activity such as those driven by eye opening or light exposure shift the modification threshold to the right – that is, stimulation frequencies that before elicited LTP now produce either no potentiation or LTD (Kirkwood et al., 1996). Conversely, rearing animals in complete darkness delays this plasticity shift, promoting LTP over LTD over a range of stimulation frequencies when compared with visually experienced animals (Kirkwood et al., 1996). Similar bidirectional and activity-dependent shifts in plasticity thresholds occur in somatosensory, piriform and motor cortices during development and learning (Quinlan et al., 2004). In most of these examples, metaplasticity is a cell-wide, global effect. Consistent with a slow time course, developmental shifts in plasticity thresholds happen gradually (over several days) and prolonged times of sensory deprivation are required to restore plasticity. Other forms of metaplasticity, however, occur in as fast as 30 minutes to 2 hours (Huang et al., 1992). For instance, the use of high-frequency stimulation 'priming' protocols in hippocampal slices triggers a rapid shift in plasticity thresholds, facilitating subsequent induction of LTD and disfavoring LTP (Wang et al., 1996).

Many different mechanisms probably contribute to metaplasticity, as is the case for synaptic plasticity. In hippocampal CA1 pyramidal and dentate gyrus cells synaptic priming requires NMDA receptor activation, suggesting that the Ca^{2+} influx through NMDA receptors is critical for the induction processes underlying both metaplasticity and synaptic plasticity (Abraham and Tate, 1997; Philpot et al., 2001). Modulations of NMDA receptor activation or the sequelae to Ca^{2+} entry are likely targets for metaplasticity expression. Thus, one can divide the possible sites of metaplasticity into two broad categories: (1) those processes, that regulate the rise in postsynaptic Ca^{2+}; and

(2) the downstream processes that are activated by the rise in intracellular Ca^{2+}. Any biochemical processes set in motion by neural activity could therefore play a role in synaptic plasticity, metaplasticity or both of them.

As a primary source of postsynaptic Ca^{2+}, NMDA receptors initiate rapid forms of synaptic plasticity, and the magnitude or duration of NMDA-receptor-mediated Ca^{2+} influx dictates the type and sign of plasticity induced. It has been shown that the magnitude and the dynamics of the Ca^{2+} transients after activation of NMDA receptors in dendritic spines determines whether the synapse will undergo LTP or LTD, with brief, large increases in spine Ca^{2+} leading to LTP and prolonged, modest increases in spine Ca^{2+} leading to LTD (Malenka and Nicoll, 1999). This reflects the smooth relationship between stimulus intensity and synaptic plasticity as demonstrated by the frequency-response relationship, where the modification threshold is the point at which the sign of the synaptic modification reverses from LTP to LTD (Bienenstock et al., 1982). For this reason, altered NMDA receptor function, and particularly changes in Ca^{2+} transients generated by NMDA receptors, have been proposed to cause metaplasticity (Shouval et al., 2002; Philpot et al., 2003).

It was demonstrated in the first part of this thesis, that Ca^{2+} through NMDA receptors leads to activation of colocalized SK channels, which limits further NMDA receptor activation. It is possible that in the absence of SK channel modulators, i.e. under basal conditions, SK channels in the hippocampus are actively suppressing memory encoding processes – thus having a modulating function consistent with the model of metaplasticity.

NR2 subunit expression is developmentally regulated such that at birth NR2B is

the predominant subunit. NR2A subunit expression begins around day 3, after which there is a gradual increase in the expression of NR2A subunits (Monyer et al., 1994; Sheng et al., 1994; Zhong et al., 1995). With maturation, the change in subunit composition results in EPSCs that have faster kinetics and are markedly less sensitive to NR2B-selective antagonists (Carmignoto and Vicini, 1992; Hestrin, 1992; Flint et al., 1997; Stocca and Vicini, 1998). This change in subunit expression is thought to contribute to the developmental changes in NMDA receptor-mediated plasticity at glutamatergic synapses (Cull-Candy et al., 2001; Philpot et al., 2001). One mechanism that could account for shifts in plasticity threshold is the developmental switch between NR2B-containing receptors, which exhibit currents with slow decay kinetics and allow the temporal summation of Ca^{2+} currents, and NR2A-containing receptors, which have faster kinetics and increased temporal resolution of Ca^{2+} signals. Philpot et al. (2003) suggested that metaplasticity might be due to a shortening of NMDA receptor-mediated EPSCs. In addition, visual experience and olfactory learning increase the proportion of NR2A-containing receptors at cortical synapses (Quinlan et al., 1999) and thus shorten the duration of NMDA receptor currents (Flint et al., 1997; Carmignoto et al., 1992; Stocca et al., 1998), correlating with a shift to higher stimulation frequencies required to produce LTP. Conversely, visual deprivation slows or reverses the switch from NR2B to NR2A (Quinlan et al., 1999).

These findings are relevant to the findings of this thesis, in that SK2 channels have been found to modulate NMDA receptor activation through their effects on the Mg^{2+} block of NMDA receptors. As a result, it is possible that a increase in the level of spine SK2 channel activity following priming stimulations would impact the NMDA

receptor EPSC, resulting in a larger SK-mediated repolarization of the spine membrane in response to synaptic stimulation.

To test the hypothesis that spine and dendritic SK channels are pathway-specifically scaled following the induction of LTP, experiments could be performed in hippocampal brain slices of wildtype and transgenic SK2 mice. Blocking SK channels, or abolishing or increasing SK2 gene expression in transgenic mice, could alter the ability of low-frequency stimulation priming to shift the subsequent frequency-response function of synaptic plasticity of field EPSPs to higher frequencies. Priming could be performed with frequencies which by themselves do not lead to changes in synaptic strength, in the absence or in the presence of SK channel blockers, and subsequently the frequency-response functions in response to conditioning stimuli could be established and compared. SK channels may regulate metaplasticity by modulating the amount of Ca^{2+} influx through NMDA receptors during priming. Priming stimulations could increase SK channel activity in dendritic spines resulting in a larger SK-mediated shunting of evoked EPSPs, contribute to a rightward shift in the modification threshold between synaptic depression and potentiation and thus towards a global decrease in dendritic excitability, whereas SK-null mice may not show metaplasticity at all.

b. Homeostatic plasticity

The term refers to the ability of neurons to maintain a preferred level of activity (spiking) in the presence of sustained alterations in synaptic stimulation. While the nervous system is inherently plastic, there are limits to this plasticity, and it has become apparent that neural activity is itself subject to homeostatic regulation to prevent neural

circuits from becoming hyper- or hypoactive. Extreme changes in network properties could disrupt ongoing function. These changes are opposed by reactive or "homeostatic" plasticity that attempts to keep function within a set range. Neurons integrate inputs from hundreds or even thousands of synaptic partners, and synapses arising from different sources can target different regions of the neuron and involve clusters of distinct receptor subtypes. Firing of each of these inputs fluctuates as a function of changes in sensory drive or internal state. Over short timescales, the activity of a central neuron must fluctuate considerably, as these fluctuations carry information. Over longer timescales, however, the same constraints apply as at the neuromuscular junction: forces that generate net increases or decreases in excitation over time will disrupt the function of central circuits if they are unopposed by homeostatic forms of synaptic plasticity.

Homeostatic forms of plasticity might provide the global negative feedback necessary to maintain synaptic strength and plasticity within a functional dynamic range, by scaling the strength of all synaptic inputs up or down while preserving their relative weights (synaptic scaling) or by altering the ability of synapses to undergo subsequent Hebbian modifications (metaplasticity) (Turrigiano et al., 1998; Abraham and Tate, 1997).

Synaptic scaling is a homeostatic form of plasticity that tends to restore neuronal activity to 'baseline' levels. It does so by changing the postsynaptic response of all of the synapses of a neuron as a function of activity. Cortical and other central neurons in culture form excitatory and inhibitory networks that develop spontaneous activity, and early studies found that blocking this activity for prolonged periods resulted in hyperactivity in these networks when activity was allowed to resume: cultured cortical

networks are composed of interconnected excitatory pyramidal and inhibitory interneurons, and develop spontaneous activity after a few days *in vitro*. This activity can be pharmacologically manipulated for long periods. Blockade of spiking activity for two days with TTX or of excitatory glutamatergic synapses with CNQX, generates a rebound phenomenon whereby the excitability of the network is increased when the drugs are removed (Turrigiano et al., 1998; Watt et al., 2000), exhibited as increased firing rate and decreased firing threshold that are due to increased average amplitude of Na^+ currents (Desai et al., 1999). Another test of the idea of firing rate homeostasis has been to raise activity with bicuculline to block $GABA_A$ receptor-mediated inhibitory transmission (Watt et al., 2000), and then to follow activity over time. After two days in bicuculline, activity has returned almost to control levels (Turrigiano et al., 1998). However, the concentrations employed (10 μM) will also block SK2 channels that have an IC_{50} of ~1 μM. Therefore the contributions of SK channels to dendritic excitability may well have been overlooked.

Most cortical synapses cluster both AMPA receptors and NMDA receptors, and the ratio of current through these two receptor types remains nearly constant after early postnatal development. Synaptic scaling was originally described for AMPA receptor-mediated postsynaptic currents. Using quantitative immunohistochemistry to measure internal and surface expression of synaptic AMPA receptors, O'Brien et al. (1998) found that activity-dependent changes in mEPSC amplitude were accompanied by changes in the accumulation of receptors at the synapse, and they demonstrated that the change in AMPA receptor number occurs through the activity-dependent regulation of receptor turnover. It is now known that NMDA receptor currents can be co-regulated with AMPA

receptor currents (Watt et al., 2000). The ratio of NMDA to AMPA current was shown to be similar at different synapses onto the same neuron, and this relationship was preserved following activity-dependent synaptic scaling. NMDA receptor scaling, as for that of AMPA receptors, occurs through changes in the number of receptors clustered at the synapse. Noise analysis suggested that the increased amplitude of NMDA-receptor-mediated mEPSCs caused by activity blockade was due to increased numbers of postsynaptic NMDA receptors rather than changes in single-channel conductance. Further, the decay kinetics and voltage-dependence of NMDA receptor currents are unaffected during scaling, also arguing against changes in NMDA receptor subunit composition. Furthermore, careful measurement of the NMDA and AMPA components of individual quantal currents has revealed a strong correlation across synapses, indicating that the ratio of current through the two receptor types is remarkably constant from synapse to synapse (Watt et al., 2000; Umemiya et al., 1999). These data show that AMPA and NMDA receptors are tightly co-regulated by activity at synapses at which they are both expressed and suggest that mechanisms exists to actively maintain a constant receptor ratio across a neuron's synapses. Functionally, maintaining a relatively constant ratio of AMPA to NMDA current might ensure that plasticity mechanisms such as synaptic scaling can normalize activity without fundamentally altering the information content of synaptic transmission.

Synaptic scaling is (1) global, because it alters the strength of the synapses of a neuron in unison, (2) gradual, because it proceeds over hours to days, and (3) multiplicative, because it scales synapses proportionally to their initial strength (Turrigiano et al., 1998). Synaptic scaling was originally described for AMPA receptor-

mediated postsynaptic currents, but it is now known that NMDA receptor currents can be co-regulated with AMPA receptor currents (Watt et al., 2000). Intriguingly, rapid forms of synaptic plasticity that selectively increase the AMPA receptor component of excitatory synaptic transmission are followed by a delayed potentiation of NMDA receptor currents (Watt et al., 2004). Thus, information regarding the weighted strength of individual synapses is preserved, as is the relative contribution of both receptor types to synaptic transmission.

These experiments, and others like them, indicate that homeostatic mechanisms adjust the cellular and synaptic properties of cortical networks to compensate for changes in synaptic drive. The reciprocal manipulation — elevating network activity by reducing a fraction of inhibition — initially raises firing rates, but over many hours firing rates fall again until they approach control levels. This shows that homeostatic regulation of firing rates can occur at the level of individual neurons in response to postsynaptic changes in activity, indicating that when cortical networks are deprived of activity, some property (or properties) of the networks is altered to promote excitability.

The finding of the first part of this thesis demonstrates that in hippocampal CA1 neurons synaptically activated SK2 channels form a Ca^{2+}-mediated feedback loop with NMDA receptors that shunts the AMPA-mediated depolarization of the spine membrane and limits Ca^{2+}-influx through NMDA receptors. Therefore, SK2 channels and NMDA receptors may form a microdomain signaling complex. SK2 channels and NMDA receptors may be co-assembled and trafficked together, and proportionate activity-dependent scaling of NMDA receptors may require SK2 channels. To test whether spine SK2 levels are scaled proportionately to NMDA receptor levels and whether dendritic

SK2 levels are decreased by excitatory blockade and increased by inhibitory blockade, one could use cultured hippocampal neurons from wildtype and transgenic SK2 mice and perform whole-cell recordings of miniature EPSCs to investigate synaptic scaling of SK2 channels, NMDA receptors and AMPA receptors following activity manipulations without or with activity blockade by TTX or activity enhancement (GABAergic block). mEPSCs recordings should reveal SK2 channel contribution to the activity-dependent proportionate scaling, blocking SK channels with apamin or using SK2-null cultures should disrupt the scaling of NMDA receptors.

c. Spike-timing-dependent plasticity

For most of this century, dendrites were considered to be passive cables. This view was shaken in the 1960s by reports of dendritic action potentials in cerebellar Purkinje cells (Llinas et al., 1968) and pyramidal neurons (Spencer and Kandel, 1961). The idea of active dendrites was slow to spread, perhaps because they were so difficult to work with (diameter < 5μm). Recently however, direct dendritic recordings, combined with imaging experiments, have shown that local action potentials can be initiated and axonally initiated action potentials can be backpropagated through the dendrites (Johnston et al., 1996; Yuste and Tank, 1996). Dendrites express just about every type of voltage-gated channel, often at different densities from the soma. These channels endow dendrites with the power to support active back-propagation of action potentials and even to generate local spikes, both of which can trigger increases in dendritic Ca^{2+} by activating dendritic voltage-gated Ca^{2+} channels (Häusser et al., 2000). Dendritic voltage-gated channels are also subject to modulation by several neurotransmitters and second-

messenger systems, thus allowing the integrative properties of dendrites to be varied over a wide range (Johnston et al., 1999). This integration is both temporal - involving the summation of stimuli that arrive in rapid succession - as well as spatial - entailing the aggregation of excitatory and inhibitory inputs from separate branches or arbors.

The dendrites of pyramidal neurons host an array of voltage-gated Na^+ and Ca^{2+}-channels that actively propagate EPSPs and local action potentials (see Magee et al., 1998 for a detailed review). Na^+ channels are distributed relatively homogeneously throughout the dendritic tree. Spatiotemporally dispersed synaptic signals result in EPSP amplitudes that are a linear reflection of the net excitatory and inhibitory synaptic inputs. If EPSPs result from highly synchronous (within a few ms) and spatially clustered (within ~100 μm) synaptic events (~50), the response becomes supralinear as a dendritic Na^+ spike is initiated close to the stimulated synapses. Alternatively, large subthreshold EPSPs, or a series of local dendritic Na^+ spikes activate voltage-gated Ca^{2+} channels and results in prolonged dendritic Ca^{2+} plateau potentials that dramatically boost the EPSPs and give rise to bursts of high frequency axonally generated action potentials. Therefore, dendritic spikes and Ca^{2+} plateau potentials provide a precise mechanism for coordinating pre- and post-synaptic activities.

Recently, a number of studies have used pairing of back-propagating action potentials and synaptic stimuli to induce synaptic plasticity (Markram et al., 1997; Magee and Johnston, 1997). These induction paradigms have aroused considerable interest because they are physiologically plausible (Zhang et al., 2000). The discovery that fast (1-2 ms), Na^+-mediated action potentials are initiated in the axon and propagate into the dendrites of hippocampal and cortical pyramidal cells (reviewed by Stuart et al., 1997),

raised the possibility that such depolarizing events might be the critical postsynaptic depolarization responsible for Hebbian plasticity, in which correlated presynaptic and postsynaptic activity strengthens synapses, whereas uncorrelated activity weakens them. In these plasticity studies, the sign of the synaptic modifications depends on the time difference between back-propagating spikes and synaptic input (Markram et al., 1997; Magee and Johnston, 1997). The Na^+-driven back-propagating action potential (or spike) reaches the fine dendritic branches and spines, opening Ca^{2+} channels along its way and causing large influxes of Ca^{2+} into dendritic shafts and spines. This way, dendritic spines, which receive the EPSPs, can also detect the spike, indicating that they are ideally poised to detect and compute temporal coincidences of neuronal inputs and outputs. In pyramidal cells of both hippocampus and cortex, close temporal pairing of synaptic activity and postsynaptic action potentials results in Ca^{2+} transients that are larger than the sum of the accumulations seen with either stimulus alone. Importantly, this supralinear summation depends on the temporal order of the two stimuli such that Ca^{2+} accumulations are larger when the postsynaptic action potentials follow the synaptic input. Supralinear accumulations of Ca^{2+} are prevented by block of NMDA receptors – therefore, NMDA receptor-mediated Ca^{2+} influx might be the signal underlying temporal coincidence detection (Schiller et al., 1998; Yuste et al., 1999). The additional Ca^{2+} influx when the spike occurs after the synaptic impulse is likely caused by the opening of NMDA receptors that have bound glutamate and from which Mg^{2+} block is relieved by the action potential (Sabatini et al., 2001). This pairing process is critical to associative long-term changes in synaptic strength and is called spike-timing-dependent plasticity (STDP).

Previous studies have shown that dendritic A-type K^+ channels modulate the induction of spike-timing dependent plasticity through NMDA receptors by affecting the success rate of back-propagating action potentials. Hoffman et al. (1997) performed a detailed study of dendritic K^+ channels in apical dendrites of CA1 pyramidal neurons from the hippocampus and found a conductance with physiological and pharmacological profiles corresponding to the A-type K^+ channels. A-type K^+ channels are low-threshold, rapidly activating and inactivating K^+ channels that increase in density with increasing distance from the soma and thus have a strong influence on action potential amplitude (Hoffman et al., 1997). Kv4.2 is likely a pore-forming subunit of A-type K^+ channels in CA1 pyramidal neurons (Cai et al., 2004), because Kv4.2 is a rapidly inactivating voltage-gated K^+ channel that activates at membrane potentials around -40 mV and is sensitive to 4-AP. These channels prevent initiation of an action potential in the dendrites, limit the back-propagation of action potentials into the dendrites, and reduce excitatory synaptic events. The channels act to prevent large, rapid dendritic depolarizations, thereby regulating orthograde and retrograde propagation of dendritic potentials. Finding a strong A-type current in apical dendrites is consistent with previous studies using intradendritic recordings and immunohistochemistry. It should be noted though that with increasing stimulus frequency, the recovery from inactivation of A-type K^+ currents is incomplete (Frick et al., 2003), thereby lessoning the influence on action potential repolarization that, if left unchecked, would affect normalizing the Ca^{2+} transient and thus reducing their role in limiting active propagation. Therefore, A-type K^+ channels act essentially as high pass filter allowing high frequency action potentials to propagate more efficiently. SK channels, on the other hand, may act as low-pass filters.

This is because SK channels are activated by Ca^{2+} influx during the action potential and, because the transient elevation of Ca^{2+} decays relatively slowly, SK channel activity is increased in each subsequent burst, thus providing the neuron with a Ca^{2+} dependent feedback mechanism for regulating synaptic integration and back-propagating action potentials in an activity-dependent manner.

It appears likely that distinct SK populations are available. Apamin-sensitive SK currents are readily recorded from CA1 pyramidal cell somas, and their inhibition has modest effects on the ability of depolarizing somatic current injections to induce fast or increased action potential discharge (e.g. Stocker et al., 1999, Bond et al., 2004). Dendritic SK channels are activated by Ca^{2+} influx through voltage-gated Ca^{2+} channels and work together with A-type K^+ channels in individual dendritic branches to shape Ca^{2+} plateau potentials (Cai et al., 2004). Cai et al. (2004) demonstrated that dendritic shaft SK channels at the site of stimulation produced a large potentiation of the ability of dendritic photolytic release of glutamate to trigger fast action potentials. Whereas dendritic plateau potentials never trigger fast action potentials in control conditions, prolongation of the plateau potential with apamin facilitated the ability of plateau potentials to trigger action potentials. Thus, SK channel activation in the dendritic shaft may participate in the responsiveness of the dendrite and may help to establish the relatively precise timing requirements for the induction of spike-timing-dependent plasticity by providing an activity-dependent shunting current that minimizes back-propagation efficiency causing a reduction in amplitude of back-propagating action potentials with distance from the soma.

Because the forms of LTP and LTD described above depend upon the activation of the NMDA receptor and subsequent Ca^{2+} entry, arrival of the back-propagating spike during the EPSP functions to depolarize the postsynaptic membrane strongly and thereby facilitate Ca^{2+} flux mediated by the NMDA receptor. In contrast, when the spike precedes the EPSP, the EPSP coincides with the afterhyperpolarization, which may dampen NMDA receptor-mediated Ca^{2+} influx. Furthermore, in hippocampal pyramidal neurons, the Ca^{2+} transient evoked by stronger stimulation is dependent on voltage-gated Ca^{2+} channels but independent of NMDA receptors, whereas the Ca^{2+} transient evoked by subthreshold stimulation is independent of voltage-gated Ca^{2+} channels and dependent on NMDA receptors (Muller and Conner, 1991). Thus, modulation of SK channels by Ca^{2+} channels and the glutamatergic system can be adjusted in an activity-dependent manner.

This leads to the hypothesis that activation of SK channels in hippocampal dendrites decreases the ability of back-propagating action potentials to invade that particular dendrite by conferring a dendritic hyperpolarization, thus (1) the amplification of action potentials by paired EPSPs will be reduced, (2) the ability of the action potentials to invade the synaptically active region will be inhibited, and (3) the incidence of pairing induced LTP will be decreased. This predicts that blocking SK channels genetically or pharmacologically would contribute to the boosting of the action potentials. This could open more Ca^{2+}-channels in the dendritic spine, thus increasing the influx of Ca^{2+} during coincident EPSPs and spikes, sufficient to induce LTP. Moreover, the larger back-propagating spike would unblock the NMDA receptors more efficiently, increasing Ca^{2+} influx into the dendritic spine. This way, the temporal coincidence of the

input and output of the cell would be detected by the effect of SK channels on the spike, and translated into a greater accumulation of Ca^{2+}.

In summary, SK channels are in an ideal position to respond to Ca^{2+} influx in response to depolarizations spreading though the dendritic cable and by acting as a braking influence on the subthreshold activation of dendritic ion channels, SK channels could function as important players in shaping the integrative properties of the dendrites. Any reduction in the availability of SK channels will increase the impact of Na^{+} and Ca^{2+} channel activation on the integration of EPSPs, reducing the level of synchrony and spatial clustering required for supralinear summation of those inputs (Yuste et al., 1997). The results discussed above strongly suggest that SK channel activity in the dendrites will have important roles in synaptic integration. One hypothesis is that SK-mediated repolarization will shape the local Ca^{2+} transients. As important regulators of dendritic function, it is possible that dendritic SK channels affect the input-output function, the efficacy of back-propagating action potentials, and local Ca^{2+} transients. In a wider sense, this could be significant because it would link three flourishing fields at the heart of cellular and systems neuroscience: LTP, intrinsic plasticity and dendritic excitability. Examining the mechanisms and functional consequences of this dendritic marriage between synaptic and intrinsic plasticity promises to lead to a deeper understanding of memory storage and dendritic function in the brain.

Dendritic recordings and Ca^{2+} imaging in brain slices could test the hypothesis of whether SK channel activity limits back-propagating action potentials. The effect of SK channel regulation on the amplitude of back-propagating action potentials after somatically evoked action potentials, as they invade the main dendritic trunk and

secondary dendrites, could be measured in the absence and presence of apamin at varying distances from the soma. It is likely that SK channels serve as a Ca^{2+}-dependent shunt that contributes to the reduction in back-propagating action potentials in dendrites, therefore blocking SK channels locally will result in an activity-dependent increase in the Ca^{2+} signal with increasing distance in dendrites. In addition, blockade of SK channels should reveal facilitation of STDP by affecting synaptically induced EPSPs, the success rate of back-propagating action potentials or locally evoked action potentials, thereby modulating the induction of synaptic plasticity.

d. AMPA receptor trafficking

Silent synapses are synapses that contain no or a very small number of AMPA receptors but an easily detectable complement of NMDA receptors. Thus they are functionally silent at normal resting membrane potentials, due to the voltage-dependent Mg^{2+}-block of the NMDA receptors. However, depolarization of the postsynaptic neuron displaces the Mg^{2+}, allowing glutamate release to induce postsynaptic responses mediated by NMDA receptors. Silent synapses are especially prevalent early in development and have been found in many brain regions, including the hippocampus, cerebral cortex, and spinal cord (Durand et al., 1996; Hsia et al. 1998, Liao et al., 1996; Wu et al., 1996). Thus, silent synapses are not a separate class of excitatory synapses that lack AMPA receptors, but rather an early stage in the ongoing maturation of the glutamatergic synapse. In the brain, soon after birth, most excitatory synapses in the hippocampus and other brain regions contain only NMDA receptors, whereas the prevalence of AMPA receptors increases gradually over the course of postnatal development (Durand et al.,

1996; Lu et al., 2001). One proposal is that early in development, a significant proportion of synapses may be functionally silent because they contain only NMDA receptors, and that over development these silent synapses become functional through an LTP-like mechanism that involves the appearance of AMPA receptors (Hsia et al., 1998). Indeed, in a number of different preparations, synaptic responses mediated solely by NMDA receptors have been observed in greatest abundance early in postnatal development (Durand et al. 1996; Isaac et al. 1997; Wu et al. 1996): electrophysiological studies have suggested that a high proportion of synapses in the brain show NMDA receptor-mediated responses without AMPA receptor-mediated responses.

The first experimental evidence for the idea that some glutamatergic synapses exhibit NMDA receptor-mediated responses in the absence of an AMPA receptor-mediated component came from a study comparing the trial-to-trial variability of AMPA- and NMDA receptor-mediated synaptic transmission at hippocampal CA1 synapses (Kullmann, 1994). Direct observation of NMDA receptor-mediated synaptic transmission in the absence of an AMPAR-mediated response came soon after (Isaac et al., 1995 and Liao et al., 1995). In these latter studies, in which minimal stimulation was used to activate one or a few synapses, two key observations were made. First, the failure rate (proportion of trials in which no synaptic response was detected) was higher at hyperpolarized holding potentials (at which NMDA receptors are blocked by Mg^{2+}) than at depolarized potentials (when NMDA receptors are unblocked). The second observation was that in a proportion of experiments no synaptic response at all was detected at hyperpolarized potentials but NMDA receptor-mediated responses were present at depolarized potentials. The simple interpretation of these findings was that some

glutamatergic synapses lack postsynaptic AMPA receptors, but contain NMDA receptors. These synapses were termed 'silent synapses'

Silent synapses are activated via the insertion of AMPA receptors into the postsynaptic membrane through an NMDA receptor-dependent LTP-like mechanism, a phenomenon commonly called 'AMPA receptor trafficking' (Kullmann, 1994, Isaac et al., 1995, Liao et al., 1995). For example, Issac et al. (1995) demonstrated that the NMDA receptor-dependent LTP at silent synapses involved the rapid appearance of AMPA receptor-mediated responses. No synaptic response was detected at a hyperpolarized holding potential (−60 mV), but, following a pairing protocol (100 stimuli at 1 Hz at a holding potential of 0 mV), AMPA receptor-mediated EPSCs were observed. It was hypothesized, that when glutamate binds to the depolarized postsynaptic cell (e.g., during Hebbian LTP), Ca^{2+} quickly enters and binds to calmodulin. Calmodulin activates Ca^{2+}-calmodulin-dependent kinase II (CaMKII), which acts on AMPA receptor-containing vesicles near the postsynaptic membrane. CaMKII phosphorylates these AMPA receptors, which serves as a signal to insert them into the postsynaptic membrane. Once AMPA receptors are inserted, the synapse is no longer silent; activated synapses no longer require simultaneous pre- and postsynaptic activity in order to elicit EPSPs. This suggests a two-step mechanism for the development of excitatory circuits: first a non-functional connection is initially formed that does not participate in basal network activity, but a second step is necessary to unsilence the connection requiring appropriate activity (Durand et al., 1996 and Isaac et al., 1995). This presents an attractive model for the activity-dependent development of neuronal circuits.

The discovery of silent synapses stimulated a major effort to determine whether AMPA receptor trafficking contributed to LTP and LTD and the molecular mechanisms responsible for activity-dependent trafficking. For LTP an important assumption of this model is that there are sufficient non-synaptic stores of AMPA receptors that can be delivered rapidly to synapses. Indeed, both light and electron microscopic studies have provided evidence for intracellular stores of AMPA receptors as well as the existence of extrasynaptic receptors in the plasma membrane (Carroll et al., 2001; Song and Huganir, 2002).

Although AMPA receptors and NMDA receptors reside in the same synapses in most brain regions, they reach their synaptic targets through quite different programs. In fact, the delivery of AMPA receptors into synapses is a regulated process that depends on NMDA receptor activation and underlies some forms of synaptic plasticity in early postnatal development and in mature neurons (Lu et al., 2001). AMPA receptor mediated responses at silent synapses can be activated following stimuli that induce NMDA receptor-dependent LTP. Therefore in silent synapses NMDA receptor activation is essential for AMPA receptor synaptic expression. The finding of this thesis, that NMDA receptors are functionally coupled to SK channels, leads to the suggestion that SK2 channels and NMDA receptors are co-assembled and trafficked together. Thus by regulating NMDA receptor activation (or strength of activation) early in development, SK channel may also influence AMPA receptor synaptic expression: SK channel activity may regulate the conversion from 'silent' (NMDA receptor only) synapses to 'functional' (AMPA- and NMDA-receptor containing) synapses in an input pattern specific and

frequency-dependent manner. Blockade of SK channel activity will lead to increases in NMDA receptor currents, thus revealing facilitation of the formation of mature synapses.

This hypothesis can be tested by recording subthreshold EPSCs, evoked by iontophoresis of 150 mM glutamate onto individual synapses, while performing whole-cell recordings from pyramidal neurons in area CA1 of the hippocampus of young mice. After stable baseline recording as measured by online analysis of the EPSC amplitude and resting membrane potential, the effect of global bath application of apamin (100 nM) compared to control conditions on evoked EPSCs could be quantified by measuring the EPSC amplitude, half time and relaxation time (time to half decay). Relieving the Mg^{2+} block of NMDA receptors by blockade of SK channel activity should result in a facilitation of AMPA receptor insertion into the postsynaptic membrane, analogous to the effect of apamin observed on the induction of synaptic plasticity (Stackman et al., 2002). It may therefore be possible that apamin-sensitive SK channels represent a neural mechanism capable of regulating NMDA receptor-dependent AMPA receptor trafficking early in development.

e. Clinical relevance: ketamine and excitotoxicity

Alterations in SK type channel activity are becoming increasingly recognized as an important control of network excitability especially in pathological states. For example, Ca^{2+} influx through L-type Ca^{2+} channels has been implicated in the initial, potentially neuroprotective, hyperpolarization in hippocampal CA3 cells in response to chemical ischemia (Tanabe et al., 1999). Clearly, the mechanisms contributing to Ca^{2+} dynamics and the activation of Ca^{2+}-dependent K^+ channels during excessive

depolarizations will be important for understanding the control of pathological neuronal hyperexcitability.

Epilepsy is a syndrome of episodic brain dysfunction characterized by recurrent unpredictable spontaneous seizures. The temporal lobe seems particularly susceptible to seizure activity. Mesial temporal lobe structures, including the hippocampus, have the lowest seizure thresholds in the brain. Conversely, thresholds in the frontal neocortex are significantly higher (Shin and McNamara, 1994). The presumed in vitro correlate of epilepsy, termed epileptiform activity, can be recorded in the hippocampal slice preparation, which provides the opportunity to investigate the cellular mechanisms underlying this disorder.

The findings of the first part of this thesis may have significant clinical relevance: Ketamine – first released for clinical use in 1970 – is an intravenous anesthetic and is still being used in a variety of clinical settings. It is a cyclohexidine derivative used for induction of anesthesia. Its mechanism of action is not well understood, but ketamine can block NMDA receptors (Adams, 1998). Ketamine is different from most other anesthetic induction agents in that it has significant analgesic effects; it does not usually depress respiratory and circulatory functions and therefore acts as a safe and reliable anesthetic (Adams, 1998). But the admissibility and limitations of this drug in patients have been discussed quickly after its introduction: ketamine can elicit seizures in patients with an epileptic diathesis – there are documented EEG-controlled cases of absence epileptic patients after ketamine administration (Kugler and Doenicke, 1994). However, the reason for epileptogenic effect of ketamine has never been established.

Considering that ketamine has proepileptogenic effects and that blockade of SK2 channels increases neuronal excitability, one could hypothesize that the epileptogenic effect of ketamine could be ascribed to an involvement of SK2 channels. The findings of the first part of this thesis propose that blocking NMDA receptors deprives SK channels of its activation by Ca^{2+}, thereby increasing excitability, yielding proepileptogenic effects, and perhaps explaining the proepileptogenic side effect of ketamine. Thus, SK channel openers and/or SK channel enhancers might represent a valuable tool to suppress network excitability.

Massive activation of glutamate receptors can result in excessive rises in cytoplasmic Ca^{2+} that are thought to underlie the fundamental processes ultimately leading to neuronal death (Choi, 1995). Preventing such cellular Ca^{2+} induced excitotoxicity in the brain may reduce considerably the neuronal damage produced by stroke, head trauma or epilepsy. Antagonist experiments suggest that most of the neuronal death associated with brief, intense glutamate exposure requires NMDA receptor activation; neuronal cell death can almost be completely inhibited by selective blockade of NMDA receptors (Bullock et al., 1992). In contrast, selective blockade of AMPA receptors has only a small effect of neuronal death (Arundine and Tyminaski, 2003). Additionally, a key role of Ca^{2+} entry in rapidly triggered excitotoxicity is supported by a quantitative correlation between the extent of cortical neuronal death induced by brief glutamate exposure and the amount of extracellular $^{45}Ca^{2+}$ that accumulates in neurons during the exposure period (Choi, 1989): $^{45}Ca^{2+}$ accumulation, like neuronal death, is mediated predominantly by NMDA receptors. This glutamate-

induced $^{45}Ca^{2+}$ accumulation can be reduced to near baseline levels by selective blockade of NMDA receptors. In contrast, only minor attenuation of degeneration or $^{45}Ca^{2+}$ influx is produced by a 100 μM concentration of the L-type Ca^{2+} channel antagonist, nifedipine.

The dependence on extracellular Ca^{2+} and NMDA receptor activation supports the hypothesis that rapidly triggered excitotoxicity is triggered by excessive Ca^{2+} influx through the Ca^{2+}-permeable NMDA receptor. Consequently, enhancing a Ca^{2+}-activated K^+ channel would exploit one of the earliest events leading to cell death. Consistant with the findings of this thesis, SK channels may therefore - in two ways - play a role in dampening glutamate excitotoxicity by suppressing the chain of events leading to CA1 pyramidal cell hyperactivity and cell death.

First, SK channels respond to rapid increases in Ca^{2+} and reduce the amplitude of excessive, NMDA receptor-mediated Ca^{2+} transients, which are a forerunner of neuronal death. SK2-mediated reinstatement of Mg^{2+} block of NMDA receptors could provide one mechanism for negative feedback regulation of activity-dependent Ca^{2+} influx into the postsynaptic spine. Such dampening of NMDA receptor activity might protect against excitotoxic cell death. By attenuating these processes, SK channels may serve to maintain the cellular homeostasis in the hippocampus.

Second, SK channels effectively modulate the number of energy-expensive action potentials (Attwell and Laughlin, 2001) discharged during depolarization by contributing to the afterhyperpolarization (Sah, 1996). Therefore, membrane hyperpolarization after activation of outwardly conducting potassium channel currents should attenuate hyperexcitation and enhance neuron survival. Enhancing SK channel function could therefore protect neurons against necrotic insults, consistent with the prediction that

enhancing SK channel function reduces excessive NMDA receptor activation, excessive cell discharge and the damaging processes that ensue.

Recently, a study was published that confirmed this hypothesis: Lappin et al. (2005) demonstrated that Ca^{2+} entry helps terminate epileptiform activity by activation of Ca^{2+}-dependent K^+-conductances in CA3 neurons of the hippocampus, highlighting the idea, that alterations in SK-type channels are becoming increasingly recognized as an important control of network excitability especially in pathological states. Clearly the mechanisms contributing to Ca^{2+} dynamics and the activation of Ca^{2+}-dependent K^+ channels during excessive depolarizations will be important for understanding the control of pathological neuronal hyperexcitability.

SK channels and neuronal excitability

Action potentials in many central neurons are followed by a prolonged afterhyperpolarization (AHP) that influences firing frequency and affects neuronal integration. Neurons transduce their synaptic inputs into repetitive firing episodes, encoding information by differences in firing frequency. Changes in the interspike interval during a repetitive firing episode or between episodes have been associated with changes in the AHPs, which reflect the underlying ionic conductances present during and after a single action potential or train of action potentials (Baldissera and Gustafsson, 1971; Lancaster and Nicoll, 1987; Schwindt et al. 1988, Lorenzon and Foehring, 1995). By shaping the neuronal firing pattern, these AHPs thus contribute to the regulation of excitability and to the encoding function of neurons.

Ca^{2+} entry during repetitive firing serves as a feedback regulator of firing rate (Wang, 1998), activating Ca^{2+}-dependent K^+ channels that produce afterhyperpolarizations and spike frequency adaptation. This feedback increases dynamic firing range and modifies information content of spike output, based on recent firing behavior (Wang, 1998). SK channels are of particular interest in these processes as they are activated by small changes in intracellular Ca^{2+} levels, thereby integrating variations in the metabolic state of the cell and in its membrane potential. Their functional role has traditionally been thought to be in controlling repetitive firing properties (Schwindt et al., 1988; Stocker et al., 1999; Abel et al., 2003; Stackman et al., 2002).

Pyramidal cells of the cerebral cortex express Ca^{2+}-activated AHPs that are mediated by a current of medium duration known as medium I_{AHP} and a slower current known as slow I_{AHP} (Schwindt et al., 1988; Stocker et al., 1999). Here, the conductances underlying the mAHP work to slow instantaneous firing rates and brake repetitive firing. In addition, the mAHP also functions to terminate short bursts of spikes (Schwindt et al., 1988). The properties of the mAHP currents, including its sensitivity to apamin and bicuculline, have identified it as being carried by channels composed of SK2 channel isoforms (Schwindt et al., 1988; Abel et al., 2003), which is in agreement with their expression profile in the anterior prefrontal–somatosensory cortices (Stocker et al., 1999).

In hippocampal CA1 pyramidal cells and many other neurons, three overlapping kinetic components of the AHP, frequently recorded as the current ascribed to the AHP (I_{AHP}) in voltage clamp, have been distinguished. Reports using pharmacological agents to examine the underlying channels have suggested that BK channels contribute to the fast I_{AHP} (I_{fAHP}) with time constants on the order of 50 ms (Lancaster and Adams, 1986;

Shao et al., 1999; Sah and Faber, 2002). A component of the medium I_{AHP} (I_{mAHP}) is eliminated by apamin, a selective blocker of SK channels, and the apamin-sensitive current decays with time constants of 200 ms (Stocker et al., 1999; Gerlach et al., 2004). BK channels, KCNQ (I_M) and HCN (I_h), also have been suggested to contribute to the medium component (Storm, 1989). The channels underlying the slow component, slow I_{AHP} (I_{sAHP}), are K^+ selective, voltage independent, and require Ca^{2+} influx through voltage-dependent Ca^{2+} channels for activation (Lancaster and Adams, 1986).

SK channels clearly contribute to the medium AHP in many neurons, as in cortical pyramidal neurons, however the role of SK channels in CA1 pyramidal neurons has been controversial. In the hippocampus, pyramidal neurons express all three subunits at different levels. Stocker et al. (1999) first demonstrated the expression of apamin-sensitive currents in CA1 neurons and showed that apamin reduced the medium AHP and increased neuronal excitability as assessed by a depolarizing somatic current step. The surprising finding was that the SK2 subunit, which is highly sensitive to apamin, was expressed at high levels in hippocampal pyramidal neurons, where no apamin-sensitive current had been described. Stocker et al. could identify a novel apamin-sensitive current mediated by SK channels in these neurons and called I_{AHP}. A key element for this finding was the discovery that this current can be blocked by bicuculline salts, traditionally used to inhibit $GABA_A$ receptors. In contrast, Storm and colleagues recently examined the AHP in CA1 neurons and found no evidence for a contribution of SK channels or other Ca^{2+}-activated K^+ channels to the mAHP, elicited by injection of a depolarizing current pulse. The intensity of the current pulse was adjusted to evoke a constant number of action potentials per pulse. Furthermore, the authors reported that there was no effect of

apamin on excitability as assessed by analysis of number, frequency or pattern of action potentials in response to a depolarizing current pulses (Gu et al., 2005).

Motivated by these results, we re-examined the role of SK channels in somatic excitability using wild type and SK2 null mice and despite earlier results could not convincingly demonstrate an effect of apamin on excitability. Blockade of SK channels did have an effect on the afterhyperpolarization elicited by trains of action potentials, but it may be hypothesized that this reflects recruitment of distant, dendritic SK channel. The marked expression in CA1 dendrites suggests that the primary roles for SK channels in CA1 neurons are regulating synaptic responses and dendritic integration.

SK channels and intrinsic plasticity

SK2 channels may also regulate hippocampal function through their effects on CA1 dendritic excitability. Activity-dependent modifications of synaptic efficacy are fundamental to the storage of information in the brain. Yet, this may not be the only way by which information can be stored – synaptic modifications are eventually summated during synaptic integration and subsequently the firing properties of the neuron become the unit of information storage. Whereas synapses mediate fast intercellular signaling in the central nervous system, information is ultimately conveyed by action potentials, which are typically initiated in the axon hillock and adjacent somatic membrane as the result of integrated synaptic activity in the dendrite and soma; increasing evidence suggests that neuronal excitability may also be modulated by activity (Saar and Barkai, 2003; Zhang and Linden, 2003).

Learning-related cellular changes can be divided into two general categories: modifications that occur at synapses and modifications in the intrinsic properties of the neurons. While it is commonly agreed that changes in strength of connections between neurons in the relevant networks underlie memory storage, it has been pointed out that modifications in intrinsic neuronal properties may also account for learning-related behavioural changes. Therefore, alterations in intrinsic excitability of a neuron are capable of encoding information as well.

Intrinsic plasticity

The intrinsic excitability of neurons has a crucial impact on the function of neural networks and is modified during the learning task in a variety of species (Alkon et al., 1985; Aizenman et al., 2003). LTP and LTD are activity-dependent, prolonged changes in the strength of synaptic connections and are thought to be a component mechanism underlying aspects of learning and memory. In addition to changes in synaptic strength, the propensity of a postsynaptic neuron to fire action potentials in response to a given EPSP is enhanced after LTP induction, suggesting a higher efficacy of EPSP-to-spike coupling. This phenomenon is known as EPSP-spike (E-S) potentiation and has been studied intensively in recent years, primarily using somatic recordings (Zhang and Linden, 2003). Most studies of plasticity have focused on changes in synaptic strength, but other potential candidates for activity-dependent changes in neuronal function include a wide array of ion channels expressed in the dendrites of these neurons (Häusser, 2004). The density and distribution of these channels largely governs the synaptic integration and neuronal excitability. This suggests that if activity could selectively modulate the

expression or the activation/inactivation state of these dendritic channels, then the integrative properties of a neuron could be markedly altered. Indeed, plasticity of intrinsic excitability may affect integration in the dendrites, soma and axon, and function together with changes in synaptic strength, thus representing a synergistic pathway for learning and memory (Häusser et al., 2004).

Several recent studies have begun to reveal mechanisms underlying intrinsic plasticity and several classes of ion channels have been implicated. There are several reports that show that hippocampal-dependent learning paradigms in behaving animals results in both increased intrinsic excitability and reduced medium and slow AHPs in CA1 (Coulter et al., 1989; Moyer et al., 1996) and CA3 (Thompson et al., 1996) pyramidal neurons, thus rendering the AHP as an important target for a variety of experience-dependent alterations in neuronal intrinsic properties. The earliest studies about experience-driven depression in AHP were described in the nudibranch mollusk *Hermissenda* by behavior training by Alkon et al. (1984). Later, they proved that a reduction of a Ca^{2+}-dependent K^+ current was responsible for these changes. In more advanced species, such as rabbits, the reduction of AHP was induced in CA1 pyramidal neurons by classic conditioning tasks. For example, Berger and his colleagues (1982) reported increased neuronal excitability in rabbit hippocampal neurons after eyeblink conditioning trials that were specific to learning. The rabbit has been a particularly useful preparation for studying associative conditioning of an eyelid-closure reflex. When a brief air puff (unconditioned stimulus) is delivered to the eye, a reflexive blink is elicited (the unconditioned response). When a neutral (conditioned) stimulus, such as a tone, is repeatedly paired with an air puff, the rabbit will learn to blink in a carefully timed

manner, such that the eyelid is closed when the air puff arrives (the conditioned response). When a trace interval is imposed between the offset of the tone and the onset of the air puff, requiring the subject to form a short-term memory of the conditioned stimulus in order to predict the unconditioned stimulus onset successfully and give a conditioned response timed appropriately to avoid the unconditioned stimulus. This task is called 'trace eyelid conditioning' and is sensitive to hippocampal inactivation. Trace eyeblink conditioning taps into the role of the hippocampal system in forming temporal associations, making the hippocampus necessary for acquiring this task (Solomon, 1979; Cohen and Eichenbaum, 1993; Wallenstein et al., 1998). The memory for trace eyeblink conditioning can last for weeks to months (Moyer et al., 1996).

Using this approach, Moyer et al. (1996) trained rabbits in trace eyelid conditioning, and prepared hippocampal slices for intracellular recording 24 hours later. They found that pyramidal neurons in areas CA1 and CA3, but not granule cells of the dentate gyrus, showed an increase in intrinsic excitability as indexed by an increase in the number of spikes evoked by depolarizing current injection, a reduction in spike accommodation during prolonged depolarizing current injection and a reduction in the amplitude of the afterhyperpolarization evoked by a spike burst. In addition, the increases in intrinsic excitability by CA1 pyramidal neurons were accompanied by a potentiation of excitatory postsynaptic potentials, demonstrating that, in conjunction with a reduction in the post-burst AHP, CA1 pyramidal neurons can respond more readily to excitatory afferents.

In the same study, voltage-clamp analysis of CA1 cells from conditioned animals showed a decrease in the Ca^{2+}-activated K^+ current I_{AHP} that is due to SK2 channel

activity (Bond et al., 2004), but not in the K^+ currents I_C or I_M, or the hyperpolarization-activated cation current I_h. This increase in intrinsic excitability was not found for animals that received unpaired tone and air puff stimulation, nor was it found in a subpopulation of animals that were unable to learn the task, in agreement with previous *in vitro* and *in vivo* data (Berger et al., 1983; Disterhoft et al., 1986, 1988). The observation that these changes persist in hippocampal slices makes it unlikely that they result from tonic activity in an extrahippocampal structure. Furthermore, conditioning-specific decreases in I_{AHP} could be recorded from voltage-clamped CA1 pyramidal neurons bathed in the Na^+-channel blocker tetrodotoxin to suppress action potentials. This result indicates that the alteration is truly intrinsic to the recorded cell and not the result of tonic synaptic drive from other neurons in the slice.

Importantly, similar reductions in hippocampal CA1 post-burst AHPs have also been observed in rats trained in an olfactory-discrimination task (Zelcer et al., 2005). In this study, rats that learned an olfactory discrimination task subsequently exhibited improved learning in the hippocampal-dependent Morris water maze task. These findings suggest that alterations of intrinsic excitability in CA1 neurons from olfactory discrimination learning effectively altered processes underlying hippocampal-dependent learning.

While there is a recent debate concerning the contributions of SK channels to the mAHP in CA1 neurons (Gu et al., 2005), SK channels clearly contribute to the mAHP in cortical neurons (Abel et al., 2004), where similar results have been found in response to operant conditioning (Saar et al., 1998). Moreover, a voltage clamp analysis of CA1 neurons showed that the medium component of the tail current, that is apamin-sensitive

(Bond et al., 2004), is reduced in rats that have learned the Morris water maze task compared to controls (Oh et al., 2003). Intriguingly, the reduction of the AHP is temporary, returning to control levels in a few days, consistent with the hypothesis that the hippocampus functions as an intermediate storage buffer during learning (Disterhoft et al., 1996). This suggests that the persistent increase in excitability facilitates modifications of synaptic strength within a time window following training, and may also be considered metaplasticity (Abraham and Bear 1996). This hypothesis has recently been extended by the observations that in addition to a transitory reduction of the mAHP and sAHP following hippocampal dependent learning, second task hippocampal-dependent learning is facilitated only during the window of opportunity when the AHP components are reduced (Zelcer et al., 2005).

Thus, it has been suggested that I_{AHP} reduction is not the mechanism by which memories are stored. Rather, it sets a time window in which activity-dependent synaptic modifications are more likely to occur. This implies that a decrease in I_{AHP} amplitude may serve as a general mechanism of increased excitability, which facilitates activity-dependent synaptic modifications that follow the Hebbian rule. Indeed, it has been shown that blocking AHP currents results in enhanced induction of LTP (Sah and Bekkers, 1996). We now appreciate that the I_{AHP} in CA1 neurons is due to SK2 channel activity (Bond et al., 2004) and therefore reducing SK channel activity may be part of the complex changes that shift the hippocampal network into a highly efficient 'learning mode', supporting the hypothesis of an involvement of Sk2 channels in metaplasticity. It is important to note that plasticity of intrinsic excitability influences the dendrites of CA1 neurons (Wang et al., 2003). As discussed above, regulation of dendritic Na^+ channels,

A-type K^+ channels, and possibly h-channels may contribute to the increased dendritic excitability. However, many of these studies have been performed in the presence of bicuculline, a blocker of both inhibitory transmission and SK channels. The findings that SK channel are prominently expressed in the dendrites (Ngo-Anh et al., 2005; Cai et al., 2004) and influence dendritic plateau potentials (Cai et al., 2004) suggest that SK channels may make their important contributions to plasticity of intrinsic excitability in the dendrites.

Taken together, the more global nature of intrinsic plasticity at a neuronal level (its ability to affect throughput from a larger number of synapses than LTP and LTD) might be useful for the adaptive generalization of non-declarative memory.

While the findings from the first part of this thesis clearly demonstrate an effect of SK channels synaptic transmission and thus explains the previous findings of an effect of SK channel blockade on hippocampal synaptic plasticity, results from the second part of this thesis and, in addition, results from the current literature also suggest the possibility that SK2 channels could modulate hippocampal intrinsic plasticity and/or hippocampal-dependent behaviours through their regulation of intrinsic dendritic excitability of CA1 neurons (Ireland et al., 2002; Sourdet et al., 2003). In cortical neurons, blockade of SK channels with apamin enhanced the temporal precision of action potential firing similar to that seen after the induction of long-term potentiation of intrinsic excitability, and activation of SK channels with EBIO reduced spike train precision (Sourdet et al., 2003). Synaptic or pharmacological activation of mGluR5 induced a persistent increase in intrinsic excitability of layer V pyramidal neurons; the increase in intrinsic excitability was blocked and occluded by apamin, indicating that downregulation of SK channels

mediates this process. Additionally, the mAHP induced after bursts of four action potentials and the I_{AHP} isolated pharmacologically were found to be depressed after induction of long-term-potentiation of intrinsic excitability with an agonist of mGluR. Several forms of activity-dependent increases in intrinsic excitability are also mediated by a downregulation of the I_{AHP}: a lasting reduction of the AHP has been reported in hippocampal neurons after classical and operant conditioning (Disterhoft et al., 1986; Saar et al., 1998).

In summary, the data from the second part of this thesis support the hypothesis that dendritic SK channels place a powerful, negative regulation on Hebbian-type association through their interaction with NMDA receptors in spines and Ca^{2+} channels in the shaft. The interactions between the AHP and NMDA receptors may be giving us an important insight into how cellular excitability changes may contribute to the pattern of specific synaptic changes occurring during learning.

Summary and conclusions

Apamin-sensitive small-conductance Ca^{2+}-activated K^+ (SK) channels regulate hippocampal synaptic responses and thus synaptic plasticity and learning.

Studies of SK channel knock out mice revealed that of the three apamin-sensitive SK channel subunits (SK1-3), only SK2 channels are necessary for the apamin-sensitive currents in CA1 pyramidal neurons. Previous results suggested that SK channel blockade with apamin, a selective SK channel antagonist, enhances the excitability of CA1 pyramidal neurons (Stocker et al., 1999; Cai et al., 2004). However, more recent results (Gu et al., 2005) and those presented in this thesis suggest that SK channels in hippocampal CA1 pyramidal neurons make, at best, a modest contribution to somatic excitability, contributing to the post-tetanus AHP at high firing frequencies. The central finding of this thesis demonstrates, that SK channels reside throughout the dendritic arbor of CA1 neurons, including the spines where they regulate synaptically evoked excitatory postsynaptic potentials (EPSPs) through their attenuation of NMDA receptor activation. During an EPSP SK2 channels in dendritic spines of CA1 pyramidal neurons are activated by increases in intracellular Ca^{2+}, resulting in repolarization of the spine membrane and reinstatement of the Mg^{2+} block of NMDA receptors, thus exerting a repolarizing, inhibitory influence upon the neuron. This SK channel-mediated feedback loop provides one mechanism, by which SK channels regulate hippocampal synaptic plasticity. These results offer a potential cellular mechanism that accounts for the previous results of Stackman et al. (2002) that showed that apamin application to hippocampal slices facilitated the acquisition if hippocampal-dependent learning tasks.

SK channels in dendritic shaft are coupled to voltage-dependent Ca^{2+} channels and regulate Ca^{2+} transients important for synaptic integration (Cai et al., 2004). It is likely that different functional roles for SK channels are endowed by distinct subcellular localizations and interactions with different microdomain components.

In conclusion, the findings of this thesis indicate that SK channels play fundamental roles in processes thought to underlie learning and memory. Storage of memories must involve some long-term physical changes in the brain. But where should we look for these changes? Depending on their subcellular location and functional coupling to various Ca^{2+} sources SK channels contribute to different forms of learning and memory.

Metaplasticity Long-term potentiation of synaptic transmission in the hippocampus is the leading experimental model for the synaptic changes that may underlie learning and memory. Interestingly, the ability of a synapse for plastic changes itself displays marked variation and plasticity. This higher form of plasticity, called "metaplasticity" can occur concurrently with synaptic plasticity via identical induction mechanisms. Here, in the first part of the thesis we report that SK channels, by affecting the degree of activation of NMDA receptors, affect the degree and direction of synaptic plasticity in the CA1 area in the hippocampus. This effect appears to be postsynaptic since apamin does not affect basal synaptic transmission, paired-pulse facilitation, and post-tetanic potentiation, as previously reported by the use of field recordings (Stackman et al., 2002). Thus, SK channels, present in dendritic spines and activated by synaptic transmission, may be a substrate for metaplasticity, encoding the recent history of synaptic plasticity and neurotransmitter modulation. This hypothesis can be investigated

by performing priming stimulations in the presence of SK channel block or enhancement and comparing the subsequent effects of conditioning pulses on the induction of synaptic plasticity.

STDP Using paired, theta frequency physiological stimulation paradigms, the direction, or sign, of synaptic plasticity depends on the temporal order in the firing of presynaptic and postsynaptic neurons. LTP requires a causal temporal relationship (pre-before post-), whereas the opposite relationship leads to LTD. An exciting new element was added to this phenomenon with a discovery by Markram et al. (1997), who used dual whole-cell patch recordings to demonstrate that the direction of synaptic modification depends on the temporal ordering, with a precision of several milliseconds. This finding was confirmed by other researchers (Bi and Poo, 1998; Sjostrom et al., 2001), and a special term was introduced to describe this type of plasticity: spike-timing-dependent plasticity (STDP). Dendritic SK2 channels may be activated by Ca^{2+} influx via voltage-gated Ca^{2+} channels and act to influence synaptic plasticity induced by "pairing". As a series of back-propagating action potentials transverse the dendrite, Ca^{2+} influx via voltage-gated Ca^{2+} channels in the dendrites may activate SK channels and blunt the synaptically evoked EPSPs, as well as limiting the success of back-propagating action potentials or locally evoked dendritic action potentials, thereby modulating the induction of synaptic plasticity. Although the functional implications of spike-timing dependent plasticity are not clear, several theoretical studies have indicated that it could underlie important effects such as sequence learning, because it tends to wire together neurons that form causal chains (Paulson and Sejnowski, 2000).

Exploring the principles that govern activity-dependent changes in excitability is an essential step to understand the function of the nervous system. I anticipate that these new insights will refine and further advance our understanding of activity-dependent synaptic modifications and thereby the mechanisms underlying behavioral learning and memory.

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