SK2 Channel Regulation of Hippocampal Function

By

Rebecca Stark Hammond

A DISSERTATION

Presented to the Department of Behavioral Neuroscience

And the Oregon Health & Science University

School of Medicine

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

September 23, 2005

School of Medicine
Oregon Health Sciences University

CERTIFICATE OF APPROVAL

This is certify that the Ph.D. thesis of

Rebecca S. Hammond

has been approved

[redacted]

John Adelman, Chair

[redacted]

Robert Stackman

[redacted]

Andrey Ryabinin

[redacted]

Kim Neve

[redacted]

James Mayne

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

There are many people I would like to thank for their support and contributions to my training and this dissertation. First, I would like to thank my mentor, Dr. Robert Stackman, for always inspiring me to be at my best, encouraging my ideas, offering me unlimited patience and support, and most importantly, teaching me scientific values through example. I would also like to thank Dr. James Maylie and Dr. John Adelman for their support and scientific training. My collaborations with John and Jim have greatly enriched my graduate studies, providing me with invaluable tools, training, and scientific dialogue. I would also like to thank Dr. Andrey Ryabinin and Dr. Kim Neve who both have taken the time to offer advice and guidance throughout my graduate studies and have contributed significantly to my training through oversight of this dissertation.

I would like to thank those who have provided me with technological training and support: Laura Tull, Dr. Chris Bond, Dr. Thanos Tzounopoulos, Eric Guire, Dr. Timothy Strassmaier, Dr. Aaron Gerlach, Thu Jennifer Ngo-Anh, Dr. Mike Lin, Keith Kohout, Sophie Davis, Julia Back, Di Fan, and Eftihia Linardatos; also, Dr. Suzanne Mitchell and Chris Kliethermes for their assistance with statistical analyses. I especially would like to thank Dr. Paco Herson who has taught me much about electrophysiology, thinking critically, and who has provided friendship and support. I am also fortunate to have had so many outstanding women to look up to during my training. For their wisdom, strength, respect and friendship, I would like to thank Dr. Anita Bechtholt, Dr. Tara Macey, Dr. Cheryl Reed-Williams, Christa Helms, Tina Gremel, Debra Peth, Kelli Kohout, Skyla Herod, and Kris Thomason.

I would like to thank my family and friends for their patience, understanding, time, love, and support. Especially Ryan Kuzmickas, who has continually made my needs and happiness a priority, and has taught me all I know about integrity, perseverance, and molecular biology. I would like to thank my parents, Robert & Denise Hammond, who have always encouraged my career goals and have been a constant source of support. Also for their encouragement and support I would like to thank Cindy & Peter Kuzmickas, Amanda & Nick Nagurney, and Jim & Chelsea Hammond.

Finally, my training would not have been possible without Char Wenger, Paul Henninger, Ginger Ashworth, or my funding sources; NIMH 1F31MH070124-01A1, NIDA 5T32DA007262, NINDS 5T32NS007466, NSF 0517689.

ABSTRACT

Deficits in declarative memory, which involve memories for events, places, and things, are common in patients suffering from Alzheimer's disease, age-related cognitive decline, epilepsy, and stroke. In humans, declarative memory processes are mediated by the medial temporal lobe, and in particular the hippocampus. The general aim of this thesis is to understand the neurobiological mechanisms of hippocampal-dependent learning and memory. In these studies, I have examined rodent models of declarative memory using a variety of behavioral and electrophysiological techniques. Specifically, my thesis work has focused on examining the role of small-conductance Ca^{2+}-activated K^+ (SK) channels in hippocampal learning and memory processes.

The first aim of this thesis (section II) was to examine the involvement of the hippocampus in object recognition memory in mice. In this study, intra-hippocampal lidocaine administration was used to inactivate the CA1 region of the dorsal hippocampus prior to training in the spontaneous object recognition task. After a 24 hour retention interval, mice administered intra-hippocampal lidocaine exhibited impaired novel object preference relative to vehicle-treated control mice. Results from this study indicate that hippocampal activation is required for object memory encoding processes. These findings are significant because they suggest that the spontaneous object recognition task is a valid paradigm for assessing rodent models of declarative memory.

The second aim of this thesis (section III) was to determine the role of SK channels in object memory processes in mice. Mice were administered apamin, a specific SK channel blocker, and nonspatial hippocampal-dependent memory was assessed using the object recognition paradigm. Systemic apamin administration enhanced object

memory encoding, but not retention processes in mice. Furthermore, intra-hippocampal apamin administration enhanced novel object preference, suggesting that *hippocampal* SK channels specifically regulate object memory. Together, results from section III are significant because 1) they suggest that SK channels modulate hippocampal-dependent memory *encoding* processes, and 2) they provide evidence that, in addition to spatial memory, SK channels also regulate nonspatial memory processes.

The final aim of this thesis was to determine the specific role of SK2 channels in hippocampal synaptic plasticity, learning, and memory. In section IV of this thesis, I examined hippocampal functions in transgenic mice that specifically overexpress SK2 channels. To test the hypothesis that SK2 channels modulate hippocampal synaptic plasticity, field excitatory postsynaptic potentials were recorded from hippocampal slices from SK2 overexpressing and wild type littermate mice. SK2 overexpression impaired long-term potentiation after 50 Hz stimulation, indicating that SK2 channels modulate hippocampal synaptic plasticity. In addition, the effects of SK2 overexpression on hippocampal-dependent behavior was examined in the Morris water maze and contextual fear conditioning paradigms. SK2 overexpression impaired learning and memory in both tasks, suggesting that SK2 channels also modulate hippocampal-dependent learning and memory processes.

Together, the studies from this thesis provide evidence that SK2 channels play an important role in the modulation of hippocampal functions. Understanding the mechanisms involved in the regulation of hippocampal processes is a necessary step in the development of novel therapies to treat memory disorders.

LIST OF TABLES AND FIGURES

ABBREVIATIONS

ACh	Acetylcholine
ACSF	Artificial cerebral spinal fluid
AHP	Afterhyperpolarization
AMPA	Alpha-amino-3hydroxy-5-methylisoxazole-4-proprionic acid
APV	2-amino-5-phosphovalerate
BK	Large conductance Ca^{2+}-activated K^+ channel
CaM	Calmodulin
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine 3',5'-monophosphate
CREB	cAMP-responsive element binding protein
DMNS	Delayed nonmatching-to-sample
DMS	Delayed matching-to-sample
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
ERK	Extracellularly regulated kinase
fAHP	Fast component of the ahfterhyperpolarization
fEPSP	Field excitatory postsynaptic potential
GABA	γ-aminobutyric acid
IAHP	Current underlying the afterhyperpolarization
IK	Intermediate conductance Ca^{2+}-activated K^+ channel
ImAHP	Apamin-sensitive current underlying the mAHP
IsAHP	Apamin-insensitive current underlying the sAHP
LTD	Long-term depression
LTP	Long-term potentiation
mAHP	Medium component of the AHP
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK activating kinase
NMDA	N-methyl-D-aspartate
PKA	cAMP-dependent protein kinase

PKC	Ca^{2+}/diacylglycerol-dependent protein kinase
PP1	Protein phosphatase 1
PP2	Protein phosphatase 2
PPF	Paired pulse facilitation
PSD	Postsynaptic density
sAHP	Slow component of the AHP
SK	Small conductance Ca^{2+}-activated K^+ channel
TEA	Tetraethylammonium
VDCC	Voltage-dependent calcium channel

I. GENERAL INTRODUCTION

Learning and Memory

Learning and memory systems

The ability to learn and remember information about the environment is vital to an organism's survival. Across species, from invertebrates to humans, many forms of learning and memory systems have evolved. In the wild, animals must learn to adapt their behavior to encountered stimuli. Simple (non-associative) forms of learning include sensitization and habituation, which involve increases or decreases (respectively) in responding to an environmental stimulus. For example, the marine snail *Aplysia californica* will eventually habituate to a repeatedly encountered innocuous stimulus (by suppressing its gill withdrawal reflex) (Pinsker, Kupfermann et al. 1970), but will sensitize to stimuli after exposure to a noxious stimulus (Pinsker, Hening et al. 1973). Animals also rely on their ability to learn associations between environmental stimuli (associative learning). With associative conditioning[1] animals can learn the predictive value of one stimulus for another. For example, a sheep receiving a foot shock directly after hearing a tone will subsequently lift its leg in response to the tone, and when placed upside-down on the shock pad will respond to the tone by lifting its head (Cahill, McGaugh et al. 2001). This example illustrates that associative conditioning involves more than a reflexive response, and environmental information is meaningfully processed during learning.

[1]Also known as classical or Pavlovian conditioning. Associative conditioning was first studied in detail in Ivan P. Pavlov's lab in the 1890s. The most famous experiment in associative conditioning involved dogs learning that the ringing of a bell predicted feeding (as measured by salivation to the sound of the bell).

In humans, learning and memory systems have been classified based on whether learned information can be consciously recollected (declarative) or not (non-declarative) (Milner, Squire et al. 1998). Declarative (or explicit) memory involves personal histories-memories for facts, events, or places that can be consciously recollected. For most people, the term 'memory' refers to declarative memory- the ability to recall your first date or simply what you ate at your last meal. On the other hand, non-declarative (or implicit) memory involves processes that are not consciously recollected, such as procedural learning. Procedural learning involves habits and skills that are unconsciously acquired. For example, learning how to tie your shoe, or how to ride a bicycle. Non-declarative memory systems also include non-associative learning, simple classical conditioning,[2] and priming.[3]

Different learning and memory systems can also be classified neuroanatomically. Lesion studies in humans and animals have shown that multiple brain regions can individually govern different learning and memory processes. For example, procedural learning is governed by the striatum (Packard, Hirsh et al. 1989; Knowlton, Mangels et al. 1996), motor learning by the cerebellum (Daum, Schugens et al. 1993; Nordholm, Thompson et al. 1993), emotional learning by the amygdala (LeDoux 1993; Cahill, Babinsky et al. 1995), and declarative memory by the medial temporal lobe (Scoville and Milner 1957; Eichenbaum 1997).

---

[2] Examples of simple classical conditioning include conditioning of muscular responses (such as eye blink conditioning), and emotional conditioning (such as fear conditioning).

[3] Priming is a learning phenomenon where previously encountered stimuli (not consciously remembered) influence subsequent performance. For example, when amnesic patients are given a list of words to remember, they are unable to consciously recall those words. However, if provided with the first few letters of a word (e.g.: r a b _ _ _, for "rabbit"), patients will be more likely to complete that word if it was present in the viewed list. (Graf, Squire et al. 1984).

As described above, declarative memory involves the conscious recollection of knowledge or experiences. At least four discrete processes are necessary for the formation, storage and recollection of declarative memories- encoding, consolidation, retention, and retrieval. *Encoding* is the first step in processing to-be-remembered information, and refers to the attending to and processing of information as it is experienced. *Consolidation* of the information occurs next, and involves the stabilization of information in preparation of memory storage. *Retention* refers to processes that are involved in the storage and maintenance of declarative memories over time. Lastly, *retrieval* involves mechanisms by which stored information is recalled.

Human declarative memory

The role of the medial temporal lobe in human declarative memory has been a popular area of research for the past 50 years, and understanding the mechanisms underlying declarative memory has remained a priority. The medial temporal lobe is an area of the brain affected by a variety of conditions, including age-related cognitive decline, senile dementia, Alzheimer's disease, epilepsy, head trauma, and stroke. In addition, amnesia for declarative memory is a common symptom of these conditions.

One of the most famous cases is of patient H.M.[4], who suffered severe amnesia after bilateral removal of the medial temporal lobes (Scoville and Milner 1957; Milner 1972). After this surgery, H.M. was unable to form new declarative memories- for example, he could not recognize doctors he interacted with daily, or remember what he had eaten for breakfast. However, H.M. did not suffer from any apparent intellectual loss,

[4] In a previous study, Milner and Penfield (1955) had reported that unilateral lesions of the medial temporal lobe impaired recent memory, but these impairments were less severe than in the case of H.M. (Milner and Penfield 1955).

and his remote memories, up until a few months prior to the surgery, remained intact. In addition, H.M. was able to meet new doctors and hold conversations, as long as his attention was not broken, indicating his immediate memory was not severely impaired. The preservation of these abilities in H.M., despite the severity of the lesion, suggested that the medial temporal lobe is involved in memory processes that occur after memory formation and prior to long-term memory storage. Through additional testing of H.M., it became apparent that non-declarative memory systems also remained intact after his surgery. For example, H.M. was able to show improvement in a mirror-drawing task. When asked to trace a star by looking at it through a mirror, errors will inevitably be made at first, but with practice, one can complete the task with few to no errors. After multiple days of training in this task, H.M.'s performance improved, even though he could not recall ever having participated in the task before. This study was one of the first to demonstrate that distinct areas of the brain are responsible for different memory systems, and the medial temporal lobe supports the declarative memory system.

The medial temporal lobe consists of multiple distinct brain regions, including the hippocampal formation, amygdala, and surrounding cortices (including the entorhinal, perirhinal, parahippocampal cortices). The importance of the hippocampus specifically in declarative memory became evident in later studies of amnesic patients with lesions restricted to the hippocampal portion of the medial temporal lobe (Zola-Morgan, Squire et al. 1986; Rempel-Clower, Zola et al. 1996). In particular, after an ischemic event, patient R.B. suffered from bilateral lesions limited to the CA1 region of the hippocampus. After this event, R.B. suffered from moderately severe memory impairment, indicating that the CA1 region of the hippocampus plays a unique role in declarative memory

processing. In addition, with the advent of magnetic resonance imaging (MRI) technology, scientists were able to examine medial temporal lobe structures in living amnesic patients. In one study, four patients with impaired declarative memory underwent MRI scans. The hippocampus was atrophied in these patients, with hippocampal volume approximately 57% of controls (Squire, Amaral et al. 1990). While these studies implicate the hippocampus as an important structure for declarative memory processes, the memory deficits of patients with restricted hippocampal damage (e.g.: R.B.) was not as severe as in those with more widespread medial temporal lobe damage (e.g.: H.M.), indicating that other structures of the medial temporal lobe also play a role in declarative memory processes.[5]

Primate Models of Declarative Memory

Declarative memory is difficult to study in animals because, by definition, it requires conscious recollection. However, lesion studies in primates and rodents have enabled researchers to examine the function of specific medial temporal lobe structures in memory processes, and to engineer behavioral tasks that model the declarative memory processes of humans. In primates, medial temporal lobe lesions similar to those H.M. experienced also resulted in similar memory impairments. This was first shown in a 1978 study (Mishkin 1978), where surgical removal of the medial temporal lobes (including

[5] While a full discussion of the contributions of extra-hippocampal structures of the medial temporal lobe is beyond the scope of this thesis, for review see: (Squire, Stark et al. 2004).

the hippocampus, amygdala, and surrounding cortices) of monkeys resulted in severe memory impairments[6].

In later studies, lesions of specific medial temporal lobe structures also revealed memory impairments in monkeys using the delayed non-matching to sample (DNMS) task (Squire and Zola-Morgan 1991), and this task was later adapted for use in rodents. In this task, animals are shown an object. After a delay they are then presented with two different objects, one from the previous trial, and one that is novel. During this choice trial, the animal is reinforced for choosing the novel object (the "non-matching" object).[7] Typically, the delay is short in this task (< 1min), and lengthening the delay increases task difficulty. Importantly, in this study the magnitude of memory impairment was dependent on which medial temporal lobe structure(s) were damaged. Monkeys with complete medial temporal lobe lesions were most severely impaired, followed by the hippocampus + parahippocampal cortex lesion group, followed by the hippocampus lesion only group, followed by the hippocampus + amygdala lesion group, and with no impairments in the amygdala lesion only group (Squire and Zola-Morgan 1991).[8] Therefore, this study supported findings from human research suggesting that the hippocampus is a necessary structure for (declarative) memory processes, and that other medial temporal lobe structures also participate in declarative memory processes.

---

[6] While hippocampal lesions in humans and primates both produced amnesic effects, there was an initial debate as to whether the behavioral tests used in primates accurately reflect the human amnesic condition (Zola-Morgan, Squire et al. 1982).

[7] In an alternative version of this task, the delayed matching to sample task (DMS), the animal is reinforced for choosing the object that was present in the previous trial.

[8] This study also demonstrated that the role of the amygdala was specific to emotional behavior, and that monkeys with amygdala lesions showed altered emotional behavior, but those with hippocampal only lesions did not.

These studies of medial temporal lobe function in primates also illustrated a broader point; that declarative memory processes could be modeled in nonverbal animals. These findings demonstrated that the biological and behavioral mechanisms of memory in primates have many similarities to those in human declarative memory, and this research has enabled the further development of rodent models of declarative memory.

Rodent Models of Declarative Memory

In the 1970s and 80s, behavioral research in rodents produced a variety of theories on the role of the hippocampus in cognitive processes. In 1978, O'Keefe and Nadel detailed their theory of hippocampal function in their book *The hippocampus as a cognitive map* (O'Keefe and Nadel 1978); and proposed that the hippocampus helps create and maintain the perception of allocentric[9] space. According to this theory, the hippocampus functions as a neural network responsible for forming a cognitive map of an environment, and the firing properties of individual hippocampal neurons represent aspects of that environment. Initially, this theory was based on findings indicating that rodents with hippocampal lesions are impaired in learning and remembering spatial information (Olton, Walker et al. 1978; Morris, Garrud et al. 1982) and that in intact animals, hippocampal neurons fire with respect to the animal's location in space (O'Keefe 1976). However, O'Keefe and Nadel's theory of hippocampal function also can be

[9] Allocentric space refers to the concept of absolute space (environmental coordinates), as opposed to egocentric space (body-centered coordinates).

broadened to include the formation of nonspatial cognitive maps- maps involving the framework by which items from an event and their interrelationships are stored[10].

Other theories of hippocampal function also arose at this time, including the *relational theory*, proposed by Howard Eichenbaum and colleagues (1986; 1988; 1989). Similar to the cognitive map theory, the relational theory suggests that the primary function of the hippocampus is to form associations between items in memory (the encoding of relational representations of items). For example, this theory predicts that place learning is compromised in rodents with hippocampal lesions, since the ability to form a mental representation of the environment (a spatial cognitive map) requires the formation of associations between those environmental stimuli. However, *the relational theory also emphasizes the role of the hippocampus in the organization, expression, and flexible use of these relationships-* properties common to declarative memory in humans (Eichenbaum 1999). Support for these aspects of the relational theory include hippocampal involvement in (1) nonspatial learning and memory functions, such as odor discrimination learning (Eichenbaum, Fagan et al. 1988; Eichenbaum, Mathews et al. 1989), (2) temporal ordering components of learning and memory (Fortin, Agster et al. 2002), (3) the flexible use of memories[11] (Eichenbaum, Stewart et al. 1990; Dusek and Eichenbaum 1998) and (4) episodic-type memory[12] (Wood, Dudchenko et al. 1999). Importantly, these studies of "human declarative-type" memory processes in rodents

[10] However, O'Keefe and Nadel still propose that these hippocampal-dependent nonspatial cognitive maps always occur within "an object spatial framework" (O'Keefe and Nadel 1978), which has led to the extensive examination of hippocampal involvement in strictly nonspatial tasks.

[11] "Flexible memory use" refers to the ability to utilize information gained in one context to solve a novel problem in another context.

[12] Episodic memory refers to memories of specific personal events. Commonly, Declarative memory in humans is divided into episodic memory and semantic memory (knowledge-based memory, facts, such as knowing your state's capitol).

demonstrate that in rodents and humans, the role of the hippocampus in memory is similar, therefore allowing for the use of rodent models of declarative memory. This thesis will address three specific tasks designed to model declarative-type memory in rodents; the Morris water maze, contextual fear conditioning, and the spontaneous object recognition task. Each of these tasks has been characterized first in rats, and later adapted for use with mice (Upchurch and Wehner 1988; Paylor, Tracy et al. 1994; Dodart, Mathis et al. 1997).

Morris Water Maze: The Morris water maze was first created to examine navigational behavior and place learning in rats with hippocampal lesions (Morris, Garrud et al. 1982). In this task, animals are trained to learn the location of a hidden platform, located in a fixed position just below the surface of the water in a circular pool. In the standard version of this task, the animal is placed into the pool from a different start location each trial and allowed to swim until reaching the hidden platform (commonly, if the animal does not locate the platform by chance within 1 min, it is placed onto the platform). Although the animal cannot see the platform, with repeated trials it will eventually learn to locate the platform relative to the position of visible cues in the room, presumably by forming a mental representation of the relationship between cues in the environment. During training, this learning is reflected by two measures, a decrease in escape latency (the time to reach the platform), and a decrease in the cumulative distance the animal is to the platform over training. In addition, probe trials in which the platform is removed from the pool can be used to assess place learning by examining the animal's search patterns. Animals that have learned the location of the hidden platform during training will exhibit

a spatial bias for the region of the pool where the platform was located during training, indicating they are searching in the appropriate region of the pool for the platform. Commonly, this spatial bias is measured by the percent time the animal spends in the target quadrant, where the platform was located during training (so that chance performance would be 25%). Probe trials are particularly useful for examination of memory retention, when administered after a long retention interval post-training. To control for possible differences in motivation, perception, and/or motor abilities between groups, a visible platform version of the Morris water maze can be utilized. In the visible task, the escape platform protrudes slightly from the water and is marked with a large visible cue (such as a flag). Rodents will quickly learn to locate this visible platform under control conditions.

Initially, Morris and colleagues (1982) found that hippocampal lesioned rats were impaired in place learning (the hidden platform version, which involves learning the platform location relative to extra-maze cues) but not cued learning (the visible platform version, which involves swimming to a visibly cued platform) in the Morris water maze. This study demonstrated that place learning in the Morris water maze is hippocampal-dependent[13], and later, other studies identified cued learning to be striatal-dependent (McDonald and White 1994). While hippocampal lesions severely disrupt learning in the Morris water maze, it was later shown that hippocampal lesions do not prevent all aspects of spatial learning. For example, with overtraining, hippocampal lesioned rats are capable of learning the platform location in the Morris water maze (Morris, Schenk et al. 1990) by employing alternative learning strategies (Eichenbaum, Stewart et al. 1990; Whishaw

[13] In this document, "hippocampal-dependent" will refer to tasks in which performance requires an intact hippocampus (i.e.: as demonstrated by lesion studies).

and Maaswinkel 1998). Rats with fornix lesions are able to locate the hidden platform when the task is altered to emphasize the use of individual associations between the platform location and extra-maze cue(s), such as calculation of swim trajectories from a constant start site (Eichenbaum, Stewart et al. 1990). Therefore, under normal conditions spatial learning in the Morris water maze reflects the formation of an associative representation of the environment (i.e.: cognitive map), similar to that observed in humans, and has become the prototypical task to examine rodent models of declarative memory.

Contextual Fear Conditioning: Fear conditioning has been used to examine learning and memory processes for many years, and the neurobiology underlying fear conditioning has been well established (for review see: (LeDoux 2000). During a fear conditioning procedure, the rodent is placed into a conditioning chamber, and receives pairings of an auditory cue (tone) and a foot shock. Subsequently, when the rodent is presented with the tone alone (in a novel context) it will exhibit its natural fear response, which is freezing (Blanchard and Blanchard 1969). This freezing to the tone demonstrates that the animal has learned the tone-shock association, and this is known as cued fear conditioning. Alternatively, if the rodent is returned to the original conditioning chamber (without the presence of the tone or shock), it will also exhibit increased freezing. This freezing to the context is termed contextual fear conditioning, and demonstrates that the animal has the ability to form, retain, and recall a mental representation of both the conditioning context and the context-shock association.

Typically, both *contextual fear conditioning* and *cued fear conditioning* are concurrently examined. Contextual fear conditioning is considered a rodent model of declarative memory because it requires the formation of a mental representation of the conditioning environment. Similar to O'Keefe and Nadel's cognitive mapping theory (1978), in contextual fear conditioning the hippocampus is necessary for the integration of environmental features into a conjunctive representation (Rudy, Huff et al. 2004). However, the amygdala, not the hippocampus, is necessary for forming associations between the mental representations of the context and shock in contextual fear conditioning, as well as associations between the mental representation of the tone and shock (LeDoux 1993) in cued fear conditioning. Therefore, cued fear conditioning is not a model of declarative memory, but is typically assessed concurrently with contextual fear conditioning to control for differences in hippocampal-independent motivational, sensorimotor, or emotional processes.

While lesions of the amygdala impair both contextual and cued fear conditioning (LeDoux 1993), hippocampal lesions selectively impair contextual (not cued) fear conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992; Kim, Rison et al. 1993). Rodents with hippocampal lesions induced prior to conditioning exhibit impaired contextual fear conditioning (Maren and Fanselow 1997; Richmond, Yee et al. 1999). In addition, contextual fear conditioning is impaired when hippocampal lesions are performed soon after (1 day) but not long after (50 days) conditioning (Anagnostaras, Maren et al. 1999). Together, these studies demonstrate that (similar to human studies) rats with hippocampal lesions suffer from both anterograde amnesia and temporally

graded retrograde amnesia, indicating that the hippocampus is required for memory processes such as encoding and consolidation, but not long-term storage.

The hippocampal-dependency of contextual fear conditioning has been historically controversial. This is due to reports that excitotoxic lesions to the dorsal hippocampus prior to training do not impair contextual fear conditioning[14] (Maren, Aharonov et al. 1997; Cho, Friedman et al. 1999; Rudy, Barrientos et al. 2002) and that hippocampal lesions do not impair contextual conditioning as measured by enhanced startle response (McNish, Gewirtz et al. 1997). However, these data do not necessarily contradict the hippocampal-dependent nature of contextual fear conditioning. In intact animals during contextual fear conditioning, a conjunctive representation of the context is formed and is associated with the foot shock, resulting in increased freezing (the fear response) to the subsequent presentation of the context. However, although hippocampal lesioned rodents cannot form and maintain a conjunctive, multi-modal representation of the context, they are still capable of learning unimodal associations between discrete cues and the foot shock. In this case, hippocampal lesioned animals will still display fear (freezing) to the conditioning context because they have learned to associate a discrete cue from the environment with the foot shock. Therefore, while intact rodents preferentially encode conjunctive representations of the context, freezing to the context may still occur in hippocampal lesioned animals that have encoded discrete feature representations of the context.

[14] It has also been argued that the observed hippocampal-dependency of contextual fear conditioning is an artifact of the hyperactivity caused by the hippocampal lesions (Richmond, Yee et al. 1999)

Spontaneous Object Recognition: As discussed above, hippocampal lesions disrupt object recognition memory in delayed matching to sample (DMS) and delayed non-matching to sample (DNMS) tasks in primates (Squire and Zola-Morgan 1991). However, in rodents the role of the hippocampus in object memory using these tasks is less clear. For example, Mumby et al. (2001) report that, on the whole, hippocampal and fornix lesions in rats do not impair object memory in DMS or DNMS tasks. However, hippocampal lesions do impair object recognition memory in a delay-dependent manner when using the spontaneous object recognition paradigm (Vnek and Rothblat 1996; Clark, Zola et al. 2000; Mumby 2001).

The spontaneous object recognition task was first characterized by Ennaceur and Delacour (1988) in rats. In this task the animal is placed into an arena with two identical objects, which the animal spontaneously explores during a sample session. After a delay, the animal is returned to the arena for a test session in which a novel object replaces one of the sample objects. If the animal has encoded information about the sample objects during the sample session and has maintained that information across the delay, then it will spend more time exploring the novel object during the test session. Therefore, this task is designed to test the recollection of object information in rodents, similar to object recollection events governed by human declarative memory. The ability of rats to recollect odor information has been demonstrated superbly by Eichenbaum and colleagues (Fortin, Wright et al. 2004). In this study, both odor recognition and odor recollection events were observed in rats, and recollection of odor information was shown to be hippocampal-dependent. The object recognition task is similarly thought to contain a hippocampal-dependent object recollection component, and therefore can be

used to assess rodent models of declarative memory. Section II of this thesis addresses the hippocampal–dependency of the spontaneous object recognition task and it's use in studying rodent models of declarative memory.

The Hippocampus

Structure

The hippocampal formation is one of the most thoroughly studied structures in the brain. The hippocampal formation includes the dentate gyrus, hippocampus, subiculum, presubiculum, parasubiculum, and the entorhinal cortex. In the rodent, the synaptic organization of the hippocampus has been well established, and therefore has become a model system for understanding the synaptic organization of neocortical structures. The rodent hippocampus is an elongated banana-shaped structure located above the thalamus, just ventral to the corpus callosum, and posterior to the septum. Both the dorsal and lateral portions of the hippocampus are bordered by parietal cortex, and the hippocampus curves in a "C" shape along its septotemporal axis. Because of its curved shape, the hippocampus was named for its resemblance to the sea horse (Greek: "hippo" -horse and "kampos" -sea monster).

Much of what we know about hippocampal neuroanatomy was deciphered by Ramon y Cajal in 1911. The hippocampus is amazingly laminar in structure, and conveniently transverse slices of the hippocampus retain their functional circuitry. Within the hippocampus, Ramon y Cajal described two distinct cell layers, the *regio inferior* and the *regio superior*. Pyramidal cells within the regio inferior have large cell bodies and receive projections from the mossy fibers of the dentate gyrus, while pyramidal cells in

the regio superior are smaller and do not receive mossy fiber input. Later, Lorente de Nó (1934) divided the hippocampus into 3 distinct regions, CA1 (regio superior), CA2, and CA3 (regio inferior)[15]. Each of these hippocampal regions is organized in the same distinct layers. 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 pyramidal cell layer. 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", is the dentate gyrus, which forms an interlocking "U" shape around the layers of CA3. The dentate gyrus itself is organized into three distinct layers. The polymorphic cell layer is located proximal to CA3, and is diffusely cellular. The granule cell layer contains the principle neurons of the dentate gyrus (granule cells). Lastly, the molecular layer is an acellular layer located adjacent to the subiculum.

Neurocircuitry

The entorhinal cortex mediates both afferent and efferent projections to and from the hippocampus. The entorhinal cortex integrates sensory information from the perirhinal and postrhinal cortices[16], and relays this information to the hippocampus via

[15] The CA2 region of the hippocampus is situated between CA1 and CA3 and contains large cells (like CA3), which do not receive mossy fiber input (like CA1). There was also a fourth region (CA4) that Lorente de Nó described. However, the CA4 region refers to the polymorphic layer of the dentate gyrus, and this term is no longer used.

[16] Perirhinal and postrhinal cortices in rodents are homologous to the parahippocampal cortex in primates.

the perforant pathway. The perforant pathway originates from layer II of the entorhinal cortex, and is so named because it "perforates" the subiculum. Perforant pathway axons terminate both in the dentate gyrus and the CA3 region of the hippocampus. The entorhinal cortex also sends projections from layer III directly to CA1 and the subiculum via the temporoammonic pathway. From the dentate gyrus, granule cells send their axons (mossy fibers) to the dendrites of CA3 pyramidal cells. CA3 pyramidal cells in turn send recurrent collaterals within CA3, and also project their axons, known as Schaffer collaterals, to the CA1 region. The CA3 to CA1 Schaffer collateral pathway also includes axons from contralateral CA3 neurons. This circuit from entorhinal cortex to dentate gyrus (perforant pathway) to CA3 (mossy fiber pathway) to CA1 (Schaffer collateral pathway) is known as the *trisynaptic pathway*, a term coined by Anderson and colleagues (1971). CA1 pyramidal cells send projections to the subiculum, lateral septum, amygdala, and back to the deep layers of the entorhinal cortex. From the subiculum, information is relayed to a variety of brain regions, including the presubiculum, parasubiculum, frontal cortex, nucleus accumbens, anterior thalamus, and medial mammillary nuclei.

The trisynaptic pathway described above is intrinsic to hippocampal function, but it is important to point out that this description is an oversimplification of hippocampal circuitry. For example, hippocampal pathways are extensively modulated by other neurotransmitter systems. These include noradrenergic inputs from the locus coeruleus, dopaminergic inputs from the substantia nigra, cholinergic and GABAergic inputs from the medial septum, and serotonergic inputs from the raphae nucleus. In addition, within the hippocampus GABAergic interneurons provide both feed-forward and feedback inhibition that is necessary for regulating excitability.

Pharmacology

Glutamate is the major excitatory neurotransmitter of the hippocampus, and both metabotropic and ionotropic glutamate receptors are found throughout the trisynaptic pathway. Metabotropic glutamate receptors are located both presynaptically (to regulate neurotransmitter release) and postsynaptically. Each of the three types of ionotropic glutamate receptors, Alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors, and kainate receptors, are found throughout the hippocampus. Each of these receptors conducts Na^+ and K^+ currents. All NMDA receptors (and some AMPA and kainate receptors) also conduct Ca^{2+}. While Na^+ through AMPA receptors mediates the fast component of the excitatory postsynaptic potential (EPSP), Ca^{2+} through NMDA receptors contributes a slow component to the EPSP (Forsythe and Westbrook 1988).

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 (Ben-Ari, Krnjevic et al. 1981) and K^+ channel modulation (Newberry and Nicoll 1984), respectively. Nicotinic acetylcholine (ACh) receptors are located presynaptically and modulate neurotransmitter release from both excitatory and inhibitory neurons (Gray, Rajan et al. 1996; Alkondon, Pereira et al. 1997). Muscarinic ACh receptors are located both presynaptically and postsynaptically. Postsynaptic muscarinic ACh receptors modulate K^+ channel conductances and the slow component of the afterhyperpolarization

following action potentials (Cole and Nicoll 1984). In addition, serotonin receptors regulate GABAergic inhibition in CA1 neurons (Ropert and Guy 1991).

Synaptic Plasticity

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. Ramon y Cajal (1911) first hypothesized that changes in synaptic connections of active neurons may play a role in information storage. Later, Hebb (1949) proposed that the mechanism for learning may involve synaptic enhancement resulting from coincident pre- and post-synaptic activity. This was directly observed by Bliss and Lømo in 1973, when they discovered that high frequency stimulation of the perforant pathway resulted in a long-term enhancement in synaptic strength, and an increase in the probability of action potential firing[17]. This phenomenon is known as long-term potentiation (LTP). LTP has since been demonstrated throughout the brain, including the CA3 and CA1 regions of the hippocampus, amygdala, cerebellum, and in a variety of cortical regions. LTP quickly became the primary cellular mechanism proposed to underlie learning and memory because it possesses similar properties to those of learning and memory; (1) LTP is long lasting, (2) LTP is easily elicited in the hippocampus- a region involved in learning and memory processes, (3) LTP is experience-dependent and input specific- so that only active synapses are capable of potentiation (McNaughton and Barnes 1977), (4) LTP is associative- it can be induced with low frequency stimulation in one pathway if paired with high frequency stimulation

[17] The increase in probability of action potential firing is also known as E-S potentiation, since there is a decreased threshold for the EPSP to elicit the firing of action potential spikes.

of another pathway (Wigstrom and Gustafsson 1986), and (5) LTP is cooperative- it usually requires the activation of multiple axons together (McNaughton, Douglas et al. 1978). In addition, LTP is readily induced by stimulation protocols that mimic the theta rhythm (Larson, Wong et al. 1986)- lending further support to the hypothesis that LTP is a mechanism of learning. The theta rhythm occurs naturally during exploratory behavior, and involves the firing of hippocampal pyramidal neurons in bursts of action potentials at a frequency range of 5-10 Hz.

In addition to LTP, synaptic transmission in multiple brain regions can undergo long-term depression (LTD). LTD involves a long-term reduction in synapse strength. Historically, synaptic depression was first observed as heterosynaptic depression in the CA1 region of the hippocampus, after an LTP-inducing stimulus was applied to an adjacent pathway (Lynch, Dunwiddie et al. 1977). *Heterosynaptic* depression involves the depression of one pathway resulting from the stimulation of another. The first observation of *homosynaptic* depression in the hippocampus occurred in a study showing the reversal of LTP with low frequency stimulation, a phenomenon now referred to as *depotentiation* (Barrionuevo, Schottler et al. 1980). Finally, homosynaptic LTD of basal transmission (*de novo*, without prior LTP) was observed in the CA1 region of the hippocampus in response to low frequency stimulation (Dudek and Bear 1992; Mulkey and Malenka 1992).

One of the problems with describing LTP and LTD is that they occur throughout the brain in many different forms. LTP and LTD are classified not only by the brain regions in which they are observed, but also by differences in mechanisms of induction, expression, and maintenance. For the purposes of this thesis, the literature review will

focus on the mechanisms of induction of NMDA receptor-dependent plasticity in the CA1 region of the hippocampus.

NMDA receptor-dependent LTP: At Schaffer collateral synapses, the induction of LTP is NMDA receptor-dependent[18]. Application of the NMDA receptor antagonist APV blocks Schaffer collateral LTP without affecting properties of baseline synaptic transmission (Collingridge, Kehl et al. 1983). NMDA receptors are vital to the induction of LTP at these synapses because they act as coincidence detectors. At resting membrane potentials, Mg^{2+} ions block the pores of NMDA receptors. Therefore, glutamate binding is not sufficient for NMDA receptor activation. However, with repetitive stimulation, current through AMPA receptors depolarizes the membrane and the Mg^{2+} block is removed, resulting in NMDA receptor activation and Ca^{2+} influx through NMDA receptors. In this manner, NMDA receptors serve as coincidence detectors, acting as sensors for the coincident activation of both pre- and post-synaptic activation. LTP is typically induced with high frequency stimulation, but can also be elicited by pairing direct postsynaptic depolarization with presynaptic low frequency stimulation. This pairing protocol is efficient at inducing LTP because the postsynaptic depolarization is sufficient for releasing the Mg^{2+} block of NMDA receptors. In addition, theta burst protocols are also commonly used to elicit LTP; these involve presynaptic stimulation in a pattern that mimics the naturally occurring theta rhythm[19].

---

[18] LTP at mossy fiber synapses is NMDAR-independent, although it is controversial whether the mechanisms underlying mossy fiber LTP are presynaptic in nature (Zalutsky and Nicoll 1990), or rely on specific postsynaptic signal transduction events (Kapur, Yeckel et al. 1998; Yeckel, Kapur et al. 1999).

[19] The most common theta burst protocol for inducing LTP *in vivo* involves trains of stimuli delivered at 20 sec intervals. Each train contains 10 bursts delivered at 5 Hz, and each burst contains 4 stimuli delivered at 100 Hz.

While NMDA receptor activation is essential for the induction of LTP in CA1, it is the subsequent rise in postsynaptic Ca^{2+} that is the trigger for LTP. LTP is inhibited by the postsynaptic application of Ca^{2+} chelators (Lynch, Larson et al. 1983). In addition, Ca^{2+} imaging studies have shown that LTP is associated with postsynaptic increases in Ca^{2+} (Regehr and Tank 1990), and that increasing postsynaptic Ca^{2+} is sufficient for LTP (Malenka, Kauer et al. 1988). NMDA receptor-*independent* LTP has also been elicited in CA1 neurons with protocols that bypass the need for NMDA receptors for elevation of postsynaptic Ca^{2+}. For example, 200 Hz stimulation of Schaffer collaterals can result in NMDA receptor-independent LTP. With this protocol, postsynaptic depolarization is large enough and long enough to open voltage-dependent Ca^{2+} channels (VDCCs), triggering increases in intracellular Ca^{2+} sufficient for LTP (Grover and Teyler 1990). In addition, NMDA receptor-independent LTP has been reported at these synapses in the presence of TEA, a nonselective K^+ channel blocker. Under these conditions, hyperexcitability from the blockade of K^+ channels enhances postsynaptic depolarization enough for sufficient increases in intracellular Ca^{2+} to trigger LTP (Aniksztejn and Ben-Ari 1991).

The induction of LTP also relies on the proper activation of Ca^{2+} trigger targets. Activation of calcium/calmodulin-dependent protein kinase II (CaMKII) is required for the induction of LTP (Pettit, Perlman et al. 1994). CAMKII is a particularly interesting target because with autophosphorylation this enzyme becomes persistently active, and may play a direct role in long-term memory storage (Lisman, Schulman et al. 2002). Calcium/phospholipid-dependent protein kinase (PKC) is also activated during the induction of LTP, and remains persistently active during the maintenance phase of LTP

(Klann, Chen et al. 1993; Sacktor, Osten et al. 1993). Other protein kinases, including cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA), mitogen-activated protein kinase (MAPK), and the tyrosine kinase Src, have also been implicated in mechanisms of LTP induction (Soderling and Derkach 2000).

The further expression of LTP is mediated by the upregulation of synaptic AMPA receptor function, which can occur by two mechanisms. First, CaMKII activated during LTP induction can phosphorylate AMPA receptors, increasing their single-channel conductance (Benke, Luthi et al. 1998; Derkach, Barria et al. 1999). Second, LTP induction can increase AMPA receptor trafficking and stabilization in dendritic spines (Malinow and Malenka 2002). Electrophysiological studies have shown that LTP induction protocols recruit AMPA receptor currents into previously "silent synapses", which are synapses that contain NMDA receptors but lack AMPA receptors (Liao, Hessler et al. 1995). In addition, activation of NMDA receptors during LTP induction triggers the redistribution of AMPA receptors into dendritic spines in hippocampal neurons (Shi, Hayashi et al. 1999). Finally, the long-term maintenance of LTP is governed by protein-synthesis dependent mechanisms (Frey, Krug et al. 1988), and involves the activation of the transcription factor cAMP-responsive element binding protein (CREB) (Bourtchuladze, Frenguelli et al. 1994; Deisseroth, Bito et al. 1996; Barco, Alarcon et al. 2002).

NMDA receptor-dependent LTD: In the CA1 region of the hippocampus, homosynaptic LTD is NMDA receptor-dependent (Dudek and Bear 1992; Mulkey and Malenka 1992). LTD is typically induced by long periods (10-15 min) of low frequency stimulation (0.5-

3Hz), but can be induced with weaker stimulation protocols if paired with postsynaptic depolarization to remove the Mg^{2+} block of NMDA receptors. Similar to LTP, LTD induction requires an increase in postsynaptic Ca^{2+}; buffering postsynaptic Ca^{2+} inhibits LTD (Mulkey and Malenka 1992), while postsynaptic uncaging of Ca^{2+} can induce LTD (Yang, Tang et al. 1999). Since increases in postsynaptic Ca^{2+} trigger both LTP and LTD, it is the properties of the Ca^{2+} signal that determine the direction of synaptic plasticity. While large increases in postsynaptic Ca^{2+} result in LTP, modest increases in postsynaptic Ca^{2+} result in LTD (Lisman 1989; Artola and Singer 1993; Malenka and Nicoll 1993). LTP inducing stimuli elicit LTD with the partial blockade of NMDA receptors using a low concentration (25μM) of APV (Cummings, Mulkey et al. 1996), supporting the hypothesis that postsynaptic Ca^{2+} concentration determines the direction of synaptic plasticity.

While LTP induction involves activation of CaMKII via Ca^{2+}/CaM, LTD induction involves the activation of the protein phosphatase calcineurin (also PP2B) via Ca^{2+}/CaM (Mulkey, Herron et al. 1993; Mulkey, Endo et al. 1994). These observations fit nicely with the Ca^{2+} concentration hypothesis of bidirectional plasticity, since calcineurin has a much higher affinity for Ca^{2+}/CaM than CaMKII (Lisman 1989; Winder and Sweatt 2001). In this model, modest increases in postsynaptic Ca^{2+} during LTD induction are sufficient to activate calcineurin pathways, but not CaMKII pathways. Once activated, calcineurin dephosphorylates and inactivates the enzyme inhibitor 1, resulting in the activation of protein phosphatase 1 (PP1) and/or protein phosphatase 2 (PP2). Activation of this phosphatase cascade can lead to the dephosphorylation of CaMKII (Strack, Barban et al. 1997) and PKA (Lee, Kameyama et al. 1998; Lee, Barbarosie et al. 2000).

Lastly, similar to LTP expression mechanisms, alterations in synaptic AMPA receptor number have also been proposed to underlie the expression of LTD. Immunocytochemical studies first showed that LTD induction results in a decrease in the number of synapses containing AMPA receptors, with no change in the number of NMDA receptor-containing synapses (Carroll, Lissin et al. 1999). This was later attributed to the rapid internalization of AMPA receptors in response to LTD-inducing stimuli (Beattie, Carroll et al. 2000).

Modulating the induction of hippocampal synaptic plasticity: Mechanisms that alter postsynaptic calcium regulate the induction of synaptic plasticity. These include molecules that regulate NMDA receptor activation. For example, there are a variety of proteins that enhance NMDA receptor activation through direct phosphorylation. These include Src family tyrosine kinases (Raymond, Tingley et al. 1994), PKC (Ben-Ari, Aniksztejn et al. 1992), PKA (Westphal, Tavalin et al. 1999), and Cyclin-dependent kinase 5 (Li, Sun et al. 2001). Proteins can also regulate NMDA receptor activation (and therefore synaptic plasticity) by contributing to the postsynaptic membrane potential (since NMDA receptor activation requires postsynaptic depolarization). For example, the inactivation properties of voltage-dependent Na^+ channels are regulated by PKC, providing a mechanism by which postsynaptic membrane potential, and subsequently NMDA receptor activation, may be regulated (Colbert and Johnston 1998). Potassium channels also play a critical role in regulating postsynaptic membrane potential. For example, "A-type" potassium channels are voltage-gated, fast-inactivating K^+ channels that repolarize the membrane after action potential firing. These channels have been

shown to regulate synaptic plasticity through their attenuation of dendritic back-propagating action potentials (Hoffman, Magee et al. 1997; Watanabe, Hoffman et al. 2002). Calcium-activated potassium channels are also located in CA1 dendrites and alter membrane potential properties. Of the calcium-activated potassium channels, small-conductance Ca^{2+}-activated K^+ (SK) channels have also been implicated in regulating hippocampal excitability (Stocker, Krause et al. 1999), synaptic plasticity (Behnisch and Reymann 1998; Stackman, Hammond et al. 2002), and learning and memory (Stackman, Hammond et al. 2002). A detailed review of this literature can be found in the following section entitled "SK channels".

Synaptic Plasticity as a Memory Mechanism

As described above, LTP and LTD are types of synaptic plasticity that have been well characterized in the hippocampus. With the examination of the Hebbian properties of synaptic plasticity in the 1950s, LTP has been thought of as the underlying mechanism of memory formation, particularly with respect to hippocampal-dependent learning and memory. Many studies support the hypothesis that LTP is a cellular correlate of hippocampal learning and memory. These include studies demonstrating that (1) pharmacological blockade of LTP impairs learning (Morris, Anderson et al. 1986; Morris 1989) (2) saturation of LTP impairs learning (Moser, Krobert et al. 1998) and (3) genetic manipulations of hippocampal LTP similarly affect hippocampal learning (Silva, Paylor et al. 1992; Silva, Wang et al. 1992; Tsien, Huerta et al. 1996; Tang, Shimizu et al. 1999). However, although the hypothesis that LTP underlies hippocampal learning and memory

has held up for the past 50 years, there continues to be a debate regarding how LTP precisely relates to mechanisms of learning and memory (Shors and Matzel 1997).

SK channels

Calcium-activated K+ currents were first identified in red blood cells (Gardos 1958), and subsequently have been observed throughout the body in most tissues, including the nervous system. Three families of Ca^{2+}-activated K^+ channels have been characterized by their varying single-channel conductances. Large-conductance (BK) Ca^{2+}-activated K^+ channels are gated by changes in membrane voltage, have a single channel conductance of 200-400 pS, are characterized by their high selectivity for K^+, and their activation is modulated by Ca^{2+} (Marty 1981). Intermediate conductance Ca^{2+}-activated K^+ (IK)[20] channels have a single channel conductance of 20-100 pS, and are activated by calcium in a voltage-independent manner (Ishii, Silvia et al. 1997; Joiner, Wang et al. 1997). Lastly, small-conductance Ca^{2+}-activated K^+ (SK) channels have a single channel conductance of 2-20 pS, and like IK channels are activated voltage-independently by increases in intracellular calcium (Blatz and Magleby 1986).

Background and Distribution

SK channels were characterized first in skeletal muscle (Romey and Lazdunski 1984), and have subsequently been observed throughout the central nervous system (Kohler, Hirschberg et al. 1996; Stocker and Pedarzani 2000; Sailer, Hu et al. 2002). Structurally, SK channels resemble voltage-gated K^+ channels, with each of their four

[20] IK channels are also known as SK4 channels.

subunits being composed of six transmembrane pore-forming domains. SK channels are activated by low concentrations of intracellular Ca^{2+} (IC_{50} = 300-700 nM) via their tight association with calmodulin. Calmodulin is constitutively bound to the C-terminal loop region of SK channel subunits, and SK channel gating is conferred by conformational changes induced by Ca^{2+} binding to these bound calmodulin molecules (Xia, Fakler et al. 1998).

Three SK channels (SK1, SK2, and SK3) have been cloned from mammalian brain (Kohler, Hirschberg et al. 1996). In the rodent brain, SK1 and SK2 channels are highly expressed in layer V of the neocortex, the subiculum, and the CA1-CA3 regions of the hippocampus (Stocker and Pedarzani 2000; Sailer, Kaufmann et al. 2004). On the other hand, SK3 channels are sparse in the hippocampus, with their highest expression in the substantia nigra, dorsal raphae, locus coeruleus and thalamus (Stocker and Pedarzani 2000; Tacconi, Carletti et al. 2001; Sailer, Kaufmann et al. 2004). It remains unclear to what extent SK channels form heteromeric channels in native tissues. However, SK channels have been observed to assemble as heteromers in heterologous expression systems (Ishii, Maylie et al. 1997; Benton, Monaghan et al. 2003), and SK2/SK3 heteromers have been coimmunoprecipitated from mouse brain membranes (Strassmaier, Bond et al. 2005), suggesting that these channels naturally heteromerize.

Pharmacology and Physiology

All three SK channel subtypes are blocked by the selective SK channel blocker apamin, a peptide derived from honey bee (*Apis mellifera*) venom (Blatz and Magleby 1986). However, SK channels are differentially sensitive to apamin, with SK2 channels

the most sensitive with IC_{50} = 63 pM (Kohler, Hirschberg et al. 1996), SK3 channels moderately sensitive with IC_{50} = 2 nM (Ishii, Maylie et al. 1997), and SK1 channels the least sensitive with IC_{50} = 8-12 nM (Shah and Haylett 2000). Consistent with this, binding studies with iodinated apamin (Mourre, Hugues et al. 1986) overlap almost identically to the expression patterns of SK2 and SK3 transcripts combined (Stocker and Pedarzani 2000). SK channels are also blocked by tamapin, tubocurarine and bicuculline methiodide (Johnson and Seutin 1997), and are activated by 1-ethyl-2-benzimidazolinone (EBIO), which enhances the calcium sensitivity of SK channels (Pedarzani, Mosbacher et al. 2001), and NS-309. However, with the exception of Lei-Dab 7, a drug which preferentially blocks SK2 channels (Shakkottai, Regaya et al. 2001), there are not sufficient pharmacological tools to examine the differential effects of SK1, SK2 and SK3 channels.

Upon elevation of intracellular calcium, SK channels activate and conduct an outward K^+ current. Consistent with this, apamin-sensitive outward K^+ currents have been observed in neurons from a variety of brain regions, including the cerebellum (Cingolani, Gymnopoulos et al. 2002), lateral amygdala (Faber and Sah 2002), subthalamic nucleus (Hallworth, Wilson et al. 2003), and hippocampus (Stocker, Krause et al. 1999). These apamin-sensitive currents participate in the afterhyperpolarization (AHP) following action potential firing. The AHP can be dissected kinetically into fast (fAHP), medium (mAHP), and slow (sAHP) components (Sah and Faber 2002). In CA1 neurons, apamin-sensitive currents (ImAHP) contribute to the mAHP (Stocker, Krause et al. 1999; Oh, Power et al. 2000; Stackman, Hammond et al. 2002; Kramar, Lin et al. 2004), although it is important to note that M-type and h-type currents also contribute to the mAHP (Storm

1989; Otmakhova and Lisman 2004; Gu, Vervaeke et al. 2005).[21] SK2 channels specifically underlie the apamin-sensitive ImAHP, since the ImAHP is absent in CA1 neurons of transgenic knockout mice lacking SK2 channels, and unaffected in CA1 neurons of knockout mice lacking SK1 or SK3 channels (Bond, Herson et al. 2004). In addition, the SK2 expression patterns throughout the brain are consistent with the distribution of the apamin-sensitive ImAHP (Stocker and Pedarzani 2000; Sailer, Hu et al. 2002). Consistent with their contribution to the mAHP, in hippocampal CA1 neurons, blockade of SK channels with apamin enhances cell excitability (Stocker, Krause et al. 1999; Stackman, Hammond et al. 2002). More recently, Cai et al. (2004) have shown that SK channels in the dendritic spines of CA1 neurons limit dendritic excitability in response to synaptic stimulation.

Role in learning, memory, and synaptic plasticity

Early observations that the hippocampal formation is rich in apamin binding sites (Mourre, Hugues et al. 1986; Mourre, Cervera et al. 1987), and that SK channels may play a role in cell excitability (Kawai and Watanabe 1986), led to the examination of an SK channel role in learning and memory. The cognitive-enhancing effects of apamin were first indicated in a bar-pressing task in mice (Messier, Mourre et al. 1991), in which administration of apamin enhanced acquisition of operant conditioning. Another study linking SK channels to learning and memory showed that immediate early gene expression (an indicator of neuronal activation) is similarly enhanced in the hippocampus

[21] BK-type currents are thought to mediate the fAHP (Lancaster and Nicoll 1987; Shao, Halvorsrud et al. 1999) and the Ca^{2+}-activated K^+ current underlying the sAHP has not yet been identified (Vogalis, Storm et al. 2003; Stocker, Hirzel et al. 2004).

of untreated operantly conditioned mice and in apamin-treated unconditioned mice (Heurteaux, Messier et al. 1993), suggesting that similar neuronal activation occurs with learning and SK channel blockade. The cognitive-enhancing effects of apamin, however, were not initially clear. For example, apamin was found to enhance learning in rats in the object recognition task when administered prior to sample session training (Deschaux, Bizot et al. 1997). However, some studies using passive avoidance (Deschaux and Bizot 1997; Ghelardini, Galeotti et al. 1998) or delayed matching-to place tasks (Poorheidari, Stanhope et al. 1998) did not find cognitive-enhancing effects of apamin. Using the Morris water maze, apamin administered prior to training was found to enhance water maze learning in mice with partial hippocampal lesions (Ikonen and Riekkinen 1999), indicating a possible role for SK channels in hippocampal-dependent learning. In a separate water maze study, mice administered apamin prior to a probe test exhibited enhanced spatial bias for the platform location (van der Staay, Fanelli et al. 1999), indicating a possible role for SK channels in hippocampal-dependent memory retention processes. However, in this latter study, apamin administered before training in the water maze had no effect on task acquisition.

Recently though (Stackman, Hammond et al. 2002), it has become clear that SK channels play a specific role in hippocampal-dependent memory encoding processes. In this study, mice were administered apamin systemically each day, 30 min prior to training in the Morris water maze. Mice were trained 4 trials/day for 6 days to learn the location of the hidden platform. After the first four trials of training, apamin-treated mice exhibited improved learning in this task, with reduced escape latency and reduced CDT compared to vehicle-treated control mice (see Appendix A, Fig. 5). Importantly, escape

latencies and CDT measures of apamin- and vehicle-treated mice did not differ on the first trial of training. In addition, in a probe test administered after the 4th training trial, apamin- but not vehicle-treated mice exhibited a strong spatial bias for the platform location, indicating that apamin-treated mice learned the platform location after 4 trials of training, and vehicle-treated control mice did not (see Appendix A, Fig. 5). A probe test administered after the 12th training trial indicated that by this point both apamin- and vehicle treated mice learned the platform location, as indicated by a strong spatial bias for the platform location. Therefore, since apamin-treated mice required less training than control mice to learn the location of the hidden platform, these results indicate that SK channels play a specific role in hippocampal-dependent memory encoding processes.

Apamin also has been shown to enhance synaptic plasticity in rodent hippocampal slices. Initially, Behnisch and Reymann (1998) found that apamin enhances the magnitude of LTP after 100 Hz stimulation. In addition, apamin was found to enhance theta rhythm amplitude in anesthetized rats, providing a possible mechanism by which SK channels may regulate learning and memory behaviors (Kinney, Patino et al. 1999). Later, Stackman et al. (2002) found that apamin enhances the induction of hippocampal synaptic plasticity (see Appendix A, Fig. 3). In this study, apamin was applied to mouse hippocampal slices, and synaptic plasticity (LTD or LTP) was examined after a variety of conditioning frequencies (1-100 Hz). The frequency-response curve for apamin and control slices was analyzed, and this curve for apamin-treated slices exhibited a leftward shift. These data indicate that LTD and LTP induction is enhanced in apamin-treated slices, since lower frequency stimulation is sufficient to induce LTP (50 Hz, versus 100 Hz), and higher frequency stimulation is sufficient to induce LTD (10Hz, versus 5 Hz).

Importantly, this study by Stackman et al. (2002) proposed an interesting mechanism by which SK channels regulate hippocampal synaptic plasticity, learning and memory. This paper proposed that during synaptic activation, increases in intracellular Ca^{2+} activate SK channels, resulting in membrane repolarization. This SK-mediated repolarization of the postsynaptic membrane in turn limits the further activation of NMDA receptors (since NMDA receptors require membrane depolarization to activate). Since synaptic plasticity in the CA1 region of the hippocampus is NMDA receptor-dependent, by regulating the activation of NMDA receptors, SK channels also regulate synaptic plasticity (and subsequently the behavioral consequences, namely, learning and memory). Recently, this hypothesis has been supported by two studies showing that SK channels regulate the NMDA receptor component of the postsynaptic EPSC in hippocampal CA1 neurons (Ngo-Anh, Bloodgood et al. 2005) and neurons of the lateral amygdala (Faber, Delaney et al. 2005), each areas where SK channels are thought to regulate synaptic plasticity, learning, and memory (Stackman, Hammond et al. 2002; Faber, Delaney et al. 2005). The general goal of this thesis is to further test the role of SK channels in hippocampal synaptic plasticity, learning and memory. The above model with respect to the present aims will be detailed in the discussion of this thesis.

Specific Aims Addressed in this Thesis

i. To determine the role of the hippocampus in object recognition memory. While lesion studies in primates and rodents suggest that the hippocampus is required for the formation of declarative memories, the role of the hippocampus specifically in object recognition memory in rodents is unclear. In some studies, hippocampal lesions disrupt

object recognition memory (Clark, Zola et al. 2000), while in others they do not (for review see (Mumby 2001). These mixed results could be due to differences in lesion techniques, resulting in varying amounts of tissue damage or possibly the recruitment of compensatory mechanisms. In addition, across these studies a variety of retention intervals were imposed. In one study, Clark et al. (2000) demonstrated that the effect of hippocampal lesions on object memory retention was delay-dependent, in that impairments in object memory were only observed with long (>1 hour) retention intervals imposed.

The first specific aim of this thesis was to determine if the hippocampus is required for object recognition memory in rodents. In this study, the hippocampus was temporarily inactivated with intra-hippocampal lidocaine administration prior to sample session training in the spontaneous object recognition task. Using this technique, we were able to inactivate the hippocampus specifically during the memory encoding/consolidation phase of the task, while also reducing the possibility of compensatory effects. In addition, two retention intervals (either short: 5 min; or long: 24 hour) were imposed in this study to further examine the delay-dependency of the hippocampal role in object recognition memory. Results from this study can be found in section II of this thesis. These findings indicate that the hippocampus indeed is involved in object recognition memory, but that this is only revealed with long (~24 hour) retention intervals imposed. These findings are significant because they validate the use of the object recognition task for the examination of hippocampal-dependent learning and memory in rodents.

ii. To determine the role of SK channels in object recognition memory. As described above, using the Morris water maze, Stackman et al. (2002) have shown that SK channels play a specific role in regulating the encoding of hippocampal-dependent memory in mice. However, it is unclear from this study alone if SK channels specifically regulate spatial hippocampal-dependent memory, or if they are involved in the regulation of multiple forms of hippocampal-dependent memories.

Therefore, aim 2 of this thesis was to determine if SK channels regulate non-spatial hippocampal-dependent learning and memory, and if their role is specific to object memory encoding or retention processes. In this study, the role of SK channels in object memory was examined using systemic administration of apamin in the spontaneous object recognition task. In addition, to examine the specific role of SK channels within the hippocampus on object recognition memory, object memory was also examined in a group of mice receiving intra-hippocampal apamin administration. Results from this study can be found in section III of this thesis. These findings indicate that SK channels specifically regulate the encoding, but not retention, of object recognition memory. These findings are significant because they support previous findings that SK channels regulate nonspatial declarative-like memory processes in rodents (Deschaux, Bizot et al. 1997), and that SK channels are specifically involved in the regulation of hippocampal-dependent memory encoding processes.

iii. To determine the role of SK2 channels in hippocampal synaptic plasticity, learning, and memory. While studies with apamin have been used to examine the role of SK channels in hippocampal functions, there are not adequate pharmacological tools to examine the specific contributions of SK1, SK2, and SK3 channels to these processes.

Recently, using transgenic knockout mice lacking either SK1, SK2, or SK3 channel subtypes, Bond et al. (2004) have shown that only SK2 channels are necessary for the apamin-sensitive ImAHP. These findings suggest that SK2 channels alone may be involved in the regulation of hippocampal synaptic plasticity, learning, and memory. In addition, this lab has recently engineered transgenic mice using gene targeting via homologous recombination to specifically overexpress SK2 channels. The development of these mice allow for the examination of the specific role of SK2 channels in hippocampal functions. Furthermore, while apamin studies have examined a gain-of-function model, with SK channel blockade resulting in enhanced hippocampal function, examination of SK2 overexpression allows for the examination of a loss-of-function model, in which it is predicted that SK2 overexpression will impair hippocampal function.

Therefore, aim 3 of this thesis was to determine the role of SK2 channels in hippocampal synaptic plasticity, learning and memory. LTP and LTD was examined in hippocampal slices from both SK2 overexpressing and wildtype littermate mice. In addition, hippocampal-dependent learning and memory was assessed in these mice using the Morris water maze and contextual fear conditioning paradigms. Results from this study can be found in section IV of this thesis. These findings indicate that SK2 channels attenuate hippocampal LTP, and impair hippocampal-dependent learning and memory. These findings are significant not only because they indicate that SK2 channels alone are capable of regulating hippocampal function, but also because they are complementary to previous studies with apamin. That is, while Stackman et al. (2002) examined gain of

hippocampal function with apamin blockade of SK channels, this study examined loss of function with the genetic overexpression of SK channels.

II. ON THE DELAY-DEPENDENT INVOLVEMENT OF THE HIPPOCAMPUS IN OBJECT RECOGNITION MEMORY

As published in Neurobiology of Learning and Memory, April 30, 2004

Abstract

The role of the hippocampus in object recognition memory processes is unclear in the current literature. Conflicting results have been found in lesion studies of both primates and rodents. Procedural differences between studies, such as retention interval, may explain these discrepancies. In the present study, acute lidocaine administration was used to temporarily inactivate the hippocampus prior to training in the spontaneous object recognition task. Male C57BL/6J mice were administered bilateral lidocaine (4%, 0.5 μl/side) or aCSF (0.5 μl/side) directly into the CA1 region of the dorsal hippocampus 5 min prior to sample object training, and object recognition memory was tested after a short (5 min) or long (24 hour) retention interval. There was no effect of intra-hippocampal lidocaine on the time needed for mice to accumulate sample object exploration, suggesting that inactivation of the hippocampus did not affect sample session activity or the motivation to explore objects. Lidocaine-treated mice exhibited impaired object recognition memory, measured as reduced novel object preference, after a 24 hour but not a 5 min retention interval. These data support a delay-dependent role for the hippocampus in object recognition memory, an effect consistent with the results of hippocampal lesion studies conducted in rats. However, these data are also consistent with the view that the hippocampus is involved in object recognition memory regardless of retention interval, and that object recognition processes of parahippocampal structures (e.g., perirhinal cortex) are sufficient to support object recognition memory over short retention intervals.

Introduction

Declarative memory in humans refers to memory for facts, events, or places, and these memories are largely dependent on the medial temporal lobe. It has been suggested that in animal models, the hippocampus is involved in aspects of human declarative memory, such as the episodic organization of stored information, as well as the flexible use of remembered information (Eichenbaum 1999). Lesions and pharmacological manipulations of the hippocampus have been shown to impair learning in mice and rats in tasks designed to model declarative memory in rodents. In rats, an intact hippocampus is required for learning the location of a hidden platform in the Morris water maze (Morris, Garrud et al. 1982), for learning contextual associations in fear conditioning tasks (Maren, Aharonov et al. 1997; McEchron, Bouwmeester et al. 1998), and for understanding temporal components of learning in trace fear conditioning tasks (McEchron, Bouwmeester et al. 1998). Hippocampal lesions produce similar impairments in spatial and nonspatial memory in mice (Chen, Kim et al. 1996; Cho, Friedman et al. 1999; Bardgett, Boeckman et al. 2003).

Object recognition memory is another model of declarative memory in which the medial temporal lobe has been implicated in primates and humans (Squire and Zola 1996). Studies of primates and rodents have shown the importance of the parahippocampal regions of the temporal lobe (namely the perirhinal, entorhinal, and inferior temporal (TE) cortices) in visual object recognition memory (Murray, Bussey et al. 2000; Gilbert and Kesner 2003). Excitotoxic lesions of the perirhinal cortex in rats disrupt object recognition memory (Aggleton, Keen et al. 1997; Liu and Bilkey 2001), and studies of neuronal activation and responses in rats and monkeys suggest it is cortical

and not hippocampal neurons that are involved in object recognition tasks (Wan, Aggleton et al. 1999; Xiang and Brown 1999; Brown and Aggleton 2001). However, some human and primate studies have shown that hippocampal lesions result in impaired object recognition memory (Cave and Squire 1991; Reed and Squire 1997; Beason-Held, Rosene et al. 1999; Zola, Squire et al. 2000), while others have shown limited effects of hippocampal lesions on object recognition memory in primates (Murray and Mishkin 1998; Baxter and Murray 2001; Zola and Squire 2001). Furthermore, studies attempting to characterize the role of the hippocampus in object recognition memory in rodents have produced inconclusive results. For example, Mumby and colleagues (1999; 2001) report that, on the whole, hippocampal or fornix lesions in rats do not impair object recognition memory in delayed matching to sample (DMS), delayed nonmatching to sample (DNMS), or spontaneous object recognition paradigms. However, in these experiments, retention intervals are short (less than 15 min), and other studies that impose longer retention intervals have found that hippocampal lesions can impair object recognition memory (Vnek and Rothblat 1996; Clark, Zola et al. 2000; Gaskin, Tremblay et al. 2003). Using the spontaneous object recognition task, Clark et al. (2000) found that rats with hippocampal lesions exhibited impaired object recognition memory with long retention intervals imposed (> 15 min) but not short retention intervals (< 15 min). However, in another study, rats with pre-training hippocampal lesions were unimpaired in an object recognition task with long (24 hr) and short (15 min) retention intervals imposed (Gaskin, Tremblay et al. 2003). It is possible that these discrepancies in the literature may be due to differences in methods of the behavioral tasks used (e.g., training

procedures, arena size, complexity of objects), or differences in the type or extensiveness of hippocampal lesions.

In addition, a number of studies have been conducted to determine the neural mechanisms underlying object recognition memory. For example, recent studies have examined the role of hippocampal N-methyl-D-aspartate (NMDA) receptors in object recognition memory. NMDA receptor activation is necessary for hippocampal long term potentiation (LTP) (Collingridge, Kehl et al. 1983), and is required for learning hippocampal-dependent tasks (Morris 1989; Davis, Butcher et al. 1992). Intra-hippocampal administration of the NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (APV) impairs object recognition memory with a long (3 hr), but not a short (5 min) retention interval (Baker and Kim 2002). In addition, transgenic mice with forebrain NMDA receptor 2B (NR2B) subunit overexpression exhibit enhanced hippocampal LTP and object recognition memory with long (1 or 3 day) retention interval imposed (Tang, Shimizu et al. 1999). Furthermore, region-specific knockout of NMDA receptor 1 (NR1) subunits in the CA1 results in impaired hippocampal LTP and impaired object recognition memory with long (>30 min) retention intervals imposed (Rampon, Tang et al. 2000). Also, elements of the mitagen-activated protein kinase (MAPK) cascade, known to be involved in hippocampal LTP and learning, are engaged in hippocampal neurons of rats tested for object recognition memory after long (24 hr) but not short (10 min) retention intervals (Blum, Moore et al. 1999; Selcher, Atkins et al. 1999; Kelly, Laroche et al. 2003). Taken together, these studies suggest that the hippocampus is involved in object recognition memory in a delay-dependent manner.

The possible delay-dependent involvement of the dorsal hippocampus in object recognition memory was examined in the present study using the spontaneous object recognition task. The spontaneous object recognition task was developed by Ennaceur and Delacour (1988) and takes advantage of rodents' natural tendency to explore novel objects, so that there is no need for food deprivation to motivate rodents to perform. In addition, unlike the historically used DMS or DNMS rodent tasks, long retention intervals ($\geq$ 24 hrs) can be successfully imposed. For these reasons, the spontaneous object recognition task has become one of the more common methods for testing object memory in rodents. Although this task has been principally characterized in rats, mice were used in this study due to the common use of transgenic mouse models in the field, which has created a need for well characterized behavioral assays of mouse hippocampal-dependent memory. Some recent studies have begun to characterize the spontaneous object recognition task in mice (Dodart, Mathis et al. 1997; Sik, van Nieuwehuyzen et al. 2003), however further characterization of mouse behavior in this task would be beneficial. Since C57BL/6J is a common background strain of transgenic mice and there is considerable data in the literature concerning their cognitive function, C57BL/6J mice were tested in this study. Hippocampal involvement in object recognition memory was tested using discrete and reversible inactivation of the dorsal hippocampus via intra-hippocampal lidocaine microinjections. This approach is advantageous because lidocaine's effects are temporary, with inactivation lasting only ~40 mins (Sandkuhler, Maisch et al. 1987). Therefore, the hippocampus can be inactivated during discrete phases of memory (e.g., encoding versus retrieval). In this study, intra-hippocampal lidocaine was administered prior to the memory encoding events (sample object

exploration), and the effects of hippocampal inactivation were tested after a short (5 min) or long (24 hr) retention interval. Inactivation of the hippocampus before the sample session impaired novel object preference 24 hr later, but not 5 min later. These results are discussed with respect to the view that there is a delay-dependent hippocampal requirement in object recognition memory in mice.

Methods

Subjects

Subjects were naïve male C57BL/6J mice 7-10 weeks old (Jackson Labs, Bar Harbor, ME). Animals were group housed and maintained in a temperature and humidity controlled vivarium on a 12-hour light/dark cycle with lights on at 0700. Food and water were available *ad libitum*. Separate cohorts of mice were used for the short retention (5 min) and the long retention (24 hr) interval studies. Each mouse received one injection only of either intra-hippocampal lidocaine (4%, 0.5 µl/side) or intra-hippocampal artificial cerebrospinal fluid (aCSF: 147mM NaCl, 2.9mM KCl, 1.6mM $MgCl_2$, 2.2mM dextrose, 1,7mM $CaCl_2 - 2H_20$, 35.9mM $NaHCO_3$, pH 7.4).

Surgery

Sixty-two C57BL/6J mice were anesthetized with 3.33 ml/kg mouse cocktail (29.4 mg/ml ketamine + 3.05 mg/ml xylazine, i.p.). Upon anesthesia, mice were secured in the stereotaxic apparatus (Cartesian Res, Sandy, OR). Burr holes were drilled bilaterally in the skull above the CA1 of the dorsal hippocampus at 2.0 mm posterior to bregma, and ± 1.5 mm lateral to bregma (Paxinos and Franklin 2001). Bilateral guide

cannulae (9.1 mm in length) were inserted 0.5 mm ventral to the cortical surface. Guide cannula were held in place with two $^{1}/_{8}$" 000 -120 jeweler's screws and acrylic dental cement. Dummy cannulae (9.1 mm, Plastics One, Inc.) were inserted into each guide cannula to prevent blockage and held in place with fitted dust caps (Plastics One, Inc.). Immediately after the surgery, mice were injected with 0.8 ml sterile saline (0.9%, i.p.) and placed in a cage on a heating pad overnight. To facilitate recovery, mice received 2 mg/ml children's liquid Tylenol in their drinking water, daily soft food, and high-calorie foods for one week prior to the start of behavioral testing. All surgeries were performed under aseptic conditions in accordance with the National Institute of Health Guidelines and were approved by the OHSU Institutional Animal Care and Use Committee.

Object Recognition Task

All mice were handled and body weights recorded in the lab for at least 2 days before conducting behavioral procedures. In all experiments, mice were habituated to a square arena (38 x 38 x 64 cm high) for 5 min/day for 2 days. The walls and floor of the arena were constructed of white acrylonitrile butadiene styrene (ABS). During each session mouse behavior was recorded with a video camera located 93.8 cm above the arena floor. This camera was interfaced with a video tracking system (EthoVision 2.3, Noldus, Leesburg, VA) that allowed us to measure velocity, cumulative distance moved, and thigmotaxis. Thigmotaxis was used as a measure of anxiety, and was calculated as the percent time spent within 6.75 cm of the arena wall. During the sample session, two identical objects (small plastic toys) were placed in opposite corners of the arena (NE and SW), approximately 2 cm from the wall. The time spent exploring each object during the

sample session was hand scored with stopwatches, and for the test session, exploration was hand scored using the EthoVision system. Exploration was defined as time spent with the head oriented towards and within 2-3 cm of the object, and with the vibrissae moving. For each experiment, the experimenter scoring mouse behavior was blind to treatment. Each mouse was removed from the arena after accumulating 38 sec of exploration time on either of the sample objects. Previous studies in our lab have found that C57BL/6J mice allowed 38 sec of sample object exploration exhibit strong novel object preference after long retention intervals (Stackman, Hammond et al. 2002). A maximum of 10 min was allotted for mice to accumulate 38 sec of object exploration during each sample session, and mice that did not reach this criterion were excluded from the study (n=3). A five min test session in which mice were placed into the arena containing one sample (familiar) object identical to that from the previous sample session and one novel object (textured metal table foot) occurred after a 5 min delay or 24 hour delay. All objects used in this study were characterized previously in pilot studies in our lab to ensure that C57BL/6J mice equally prefer sample and test session objects. Novel and familiar object location was counterbalanced across animals. Mice were placed into a polycarbonate mouse cage for 5 min prior to each session in the arena (habituation, sample, and test sessions). After each session the arena and objects were cleaned thoroughly with 10% ethanol to ensure that behavior of the mice was not guided by odor cues.

Intra-Hippocampal Infusions

To habituate mice to the intra-cranial microinfusion process, mice were gently restrained and dummy cannulae were removed and replaced for three days. On day one, dummy cannulae were removed and replaced only. On day two, dummy cannulae were removed and replaced, followed by a 5 min arena habituation session. On day three, dummy cannulae were removed and an empty infusion cannula was inserted through each guide cannula into the hippocampus for 3 min. Dummy cannulae were replaced just prior to a final 5 min arena habituation session. On day four, each mouse received bilateral (0.5 µl/side) intra-hippocampal infusions of aCSF or lidocaine (4% in aCSF; Sigma, St. Louis, MO) at a rate of 0.33 µl/min. Intra-hippocampal infusions were performed with 9.6 mm infusion cannula (Plastics One, Inc.) connected via 20 gauge polyethylene tubing to 10 µl Hamilton syringes secured in a Razel motorized syringe pump. Infusion cannulae were left in place for 90 sec following completion of the bilateral intra-hippocampal infusion. Upon removal of the infusion cannulae, dummy cannulae were replaced prior to the sample session.

Histology

To verify cannulae placement, brain tissue from each mouse was sectioned, stained and analyzed. Mice were euthanized with an overdose of mouse cocktail (29.4 mg/ml ketamine + 3.05 mg/ml xylazine, i.p.). The brains were carefully removed and transferred to 4% paraformaldehyde in a phosphate buffered saline solution (PBS; 10 mM, pH = 7.4) for a minimum of 24 hours. For cryoprotection, each brain was transferred and equilibrated to a 20% then 30% sucrose solution (in PBS). Each brain was

sectioned (40 μm), mounted, and stained with Cresyl violet. Cannulae placement was determined using a light microscope (Zeiss Axioplan, West Germany). Images of microinjection sites were recorded with a Polaroid digital microscope camera and Polaroid DMC direct software. For a subset of animals, thionin (0.5 μl/side) was injected into the hippocampus just prior to euthanasia to examine the range of diffusion in these studies. Histological analysis was carried out as described above, except brain slices were background stained with neutral red to allow optimal visualization of thionin dye. Figure 1 shows the placement of guide cannula in three representative animals. Behavioral data from 14 mice were excluded due to poor cannula placement or poor histological processing of the tissue. For mice that received thionin injections, no thionin dye was detected in extrahippocampal structures (see Fig. 2.1c for a representative section).

Data Analysis

Differences in latencies to accumulate 38 sec object exploration during the sample session were analyzed using an independent groups Student's *t*-test. For each experiment, novel object preference was expressed as a *preference ratio*, which was calculated by dividing the amount of exploration of the novel object by the total amount of object exploration during the test session. Therefore, a preference ratio above 0.5 would indicate novel object preference, below 0.5 familiar object preference, or equal to 0.5 no preference (chance performance). Student's *t*-tests were used to verify that novel object preference was above chance (0.5), as well as to compare novel object preference between groups. A *difference score* was also determined for each mouse by subtracting the amount of time spent exploring the novel object from that spent exploring the familiar

object during the test session. To examine possible differences in motor activity or motivation between treatment conditions, velocity, thigmotaxis (percent time spent within 6.75 cm of the arena wall) and the total distance moved were examined. A 4% lidocaine injection produces an inactivation of neural tissue lasting ~40 min (Sandkuhler, Maisch et al. 1987). Thus, dorsal hippocampal activity was suppressed during both the sample *and* test session in those mice tested at the short retention interval, but was only suppressed during the sample session in the mice tested with the long retention interval. Therefore, noncognitive performance measures were each analyzed with a two-factor (one between subjects variable: treatment; and one within subjects variable: session) ANOVA for the short retention interval study and with an independent groups Student's *t*-test for the long retention interval study.

Results

Intra-hippocampal lidocaine administration with short (5 min) delay object recognition.

Lidocaine (n = 14) or aCSF (n = 10) was infused bilaterally into the dorsal hippocampus 5 min before the sample session and object memory retention was tested after a 5 min retention interval. During the sample session, there was no effect of treatment on the latency to accumulate 38 sec of sample object exploration (t (22) = 0.238; $P > 0.05$), suggesting that all mice were equally motivated to explore objects (Table 1). In addition, each group exhibited novel object preference during the test session (aCSF: t (9) = 7.081; $P < 0.001$; Lido: t (13) = 6.668; $P < 0.001$), with no significant difference between treatment groups in novel object preference as measured by the preference ratio (Fig. 2.2, t (22) = 0.775; $P > 0.05$) or the difference score (data not

shown, t (22) = 1.629; $P > 0.05$), suggesting that hippocampal activity is not required for short delay object recognition memory.

Several performance measures were analyzed to determine whether intra-hippocampal lidocaine administration disrupts motor activity or anxiety in these mice (Table 2.1). Due to the short (5 min) retention interval, lidocaine was present in the hippocampus for the sample and test sessions. Therefore, noncognitive performance measures were analyzed across the sample and test sessions. Repeated measures ANOVAs found no effect of lidocaine treatment on velocity (F (1,22) = 2.218; $P > 0.05$), total distance moved, (F (1,22) =1.976; $P > 0.05$) or thigmotaxis (F (1,22) = 0.896; $P > 0.05$) across the sample and test sessions, indicating that intra-hippocampal lidocaine administration does not significantly affect these noncognitive performance measures.

Intra-hippocampal lidocaine administration with long (24 hr) delay object recognition.

As in the previous experiment, lidocaine (n = 9) or aCSF (n = 12) was infused bilaterally into the hippocampus of naïve C57BL/6J mice 5 min before the sample session. A test session presented after a 24-hour retention interval assessed object memory retention. During the sample session, there was no effect of treatment on the latency to accumulate 38 sec of sample object exploration (t (19) = -0.719; $P > 0.05$), indicating that all mice were equally motivated to explore objects (Table 2.1). In addition, each group exhibited novel object preference during the test session (aCSF: t (11) = 9.916; $P < 0.001$; Lido: t (8) = 6.744; $P < 0.001$), however, mice administered intra-hippocampal lidocaine exhibited significantly less novel object preference during the test session than aCSF-treated control mice. This significant difference in novel object

preference was found with both measures, the preference ratio (Fig 2.2, t (19) = 3.108; P = 0.006), and the difference score (data not shown, t (19) = 2.822; P = 0.011). These results indicate that hippocampal activity is required for the encoding of object recognition memory and/or for retaining object recognition memory over a long (24 hr) delay.

Several noncognitive performance measures were analyzed to determine whether differences in sensorimotor ability or anxiety could have contributed to poor object memory encoding in lidocaine-treated mice during the sample session, when lidocaine was present in the hippocampus (Table 2.1). No difference between treatment conditions were found in velocity (t (19) = -0.460; $P > 0.05$), total distance moved (t (19) = -1.261; $P > 0.05$), or thigmotaxis (t (19) = -0.983; $P > 0.05$) during the sample session. These results indicate that the memory impairing effect of intra-hippocampal lidocaine is not confounded by effects of lidocaine on these noncognitive performance measures.

Discussion

Results of the short delay experiment suggest that the hippocampus *is not* required for object memory encoding and/or retrieval with short retention intervals, since hippocampal neural inactivation with lidocaine pretreatment does not impair novel object preference after a 5 min retention interval. However, it should be noted that in Experiment 1, the CA1 region of the dorsal hippocampus was inactivated during the sample training session, retention interval, and the testing session. This design does not allow us to explicitly examine the hippocampal role in object memory encoding, since differences observed during the test session could also be due to effects on consolidation and/or retrieval. Regardless, there was no impairment found, suggesting that the dorsal hippocampus is not necessary for object memory processes when short retention intervals are imposed. This result is consistent with previous studies of hippocampal-lesioned rats (Mumby 2001).

For the long delay experiment, the results suggest that the hippocampus *is* involved in the encoding of object memory, since neural inactivation of the hippocampus with lidocaine prior to the sample session impairs novel object preference after a 24-hour retention interval. The lidocaine-induced impairment in object memory encoding seen in this experiment is unlikely to be due to noncognitive effects of the drug during the sample session. There were no differences between treatment groups in the latency to accumulate sample object exploration or other measures of motor activity, suggesting that intra-hippocampal lidocaine administration did not have significant sensory, motor, or motivational influences. Although intra-hippocampal lidocaine administration did not completely block object memory encoding, which would be indicated by a preference

ratio of 0.5 (chance performance), the lidocaine-induced impairment illustrated in Fig. 2.2 is significant, especially when considering that the injections were restricted to a small portion of the CA1 region of the dorsal hippocampus, leaving some dorsal and most ventral areas of the hippocampus active. These results are not likely to be due to lidocaine inactivating surrounding cortical tissue since the injection volume is small (0.5 µl/side) and only animals with cannula placement verified at CA1 were included in the results.

Neural inactivation with intra-hippocampal lidocaine

To verify the effect of discrete inactivation of dorsal CA1 on hippocampal function, a separate study was conducted in our lab in which lidocaine was shown to impair spatial learning in the Morris water maze task. In this experiment, male C57BL/6J mice received bilateral intra-hippocampal injections of lidocaine (4%) or aCSF daily, prior to each 4-trial block of training. Lidocaine treatment significantly impaired acquisition of the Morris water maze task, as measured by the cumulative distance of the mouse to the platform (F (1,33) = 5.256; P = 0.028). This result indicates that the discrete inactivation of the dorsal CA1 region of the hippocampus impairs spatial memory in C57BL/6J mice. Thus, inactivating a relatively small area of the dorsal hippocampus yields a significant affect on learning, a finding consistent with other studies (Moser, Moser et al. 1995; Riedel, Micheau et al. 1999; Corcoran and Maren 2001; Lee and Kesner 2003).

A major advantage to lidocaine-induced neural inactivation is that the effects are temporary. Traditional permanent lesions (e.g., electrolytic, excitotoxic) of the

hippocampus may result in compensatory changes in the surrounding neural circuitry, making it difficult to attribute behavioral effects to the loss of the missing structure (Clusmann, Nitsch et al. 1994; Cassel, Duconseille et al. 1997). Temporary neural inactivation with intra-hippocampal lidocaine administration can be utilized to assess the role of hippocampal activity in object recognition memory while avoiding long-term compensatory changes. In addition, these temporary lidocaine "lesions" allow for the testing of hippocampal involvement in discrete memory processes. In the long delay experiment of the present study, the dorsal hippocampus was inactivated during the sample session, but was functional during the test session 24 hours later. Therefore, results from this experiment suggest that hippocampal activation is involved specifically in early object memory processes (such as encoding or early time points of consolidation). In future studies, administering intra-hippocampal lidocaine at various time points after the sample session would permit testing the involvement of the hippocampus in specific object memory processes (e.g., consolidation, retrieval) and the relative timing of these processes. It would also be interesting to further investigate the role of the hippocampus in reconsolidation processes of object recognition memory. In a recent study examining the role of MAPK in object recognition memory, it was shown that i.c.v. administration of MEK inhibitor UO126 prior to reactivation of object memories impaired novel object preference in rats, and during reconsolidation in this task increases in ERK phosphorylation in the CA1 region of the hippocampus were observed (Kelly, Laroche et al. 2003).

Hippocampal involvement in object (recognition) memory

Together, findings from the present study and the current literature suggest a delay-dependent involvement of the hippocampus in object recognition memory in mice, an effect consistent with most studies in rats. The possibility of species differences when comparing mouse and rat studies should be noted though, and may explain how in a recent study, no impairment in long delay (24 hr) object recognition memory was found in rats with hippocampal lesions (Gaskin, Tremblay et al. 2003). After hippocampal or fornix lesions in rats, object recognition memory remains intact when tested after short (< 15 min) retention intervals (Mumby 2001; Mumby, Gaskin et al. 2002), and other studies with long retention intervals have shown there is hippocampal involvement in object recognition memory (Vnek and Rothblat 1996; Clark, Zola et al. 2000). Although these studies *observe* delay-dependent hippocampal involvement in object recognition memory, we hypothesize that the hippocampus is active in object memory encoding regardless of retention interval. The fact that hippocampal lesions do not affect object recognition memory with short retention intervals may be due to the parallel involvement of temporal cortical structures. In the present study, the role of the hippocampus in long-delay object recognition memory is shown to be restricted to object memory *encoding* or early processes of *consolidation*. If the hippocampus is involved in object memory encoding, but only when a long retention interval is imposed, then the hippocampus would need to anticipate the duration of the retention interval during memory encoding. It is more likely that regardless of the retention interval, lesions of the hippocampus impair object memory encoding, and no impairment is observed in lesioned animals with short retention intervals imposed because parahippocampal structures involved in object

recognition processes (e.g., perirhinal cortex) are sufficient to support short-term object recognition memory. We hypothesize that the hippocampus and the perirhinal cortex play very different roles in object recognition memory. For example, the perirhinal cortex may be necessary for encoding basic information about an object's familiarity/novelty ("object recognition"), while the hippocampus is involved in encoding information about the experience of the object ("object memory"). In fact, various studies of object recognition in rodents and non human primates have established that the perirhinal cortex is critical for object recognition, and that patterns of neuronal activation in this region have been suggested to code for object familiarity (Zhu, McCabe et al. 1996; Xiang and Brown 1999; Bussey, Duck et al. 2000; Gaffan, Eacott et al. 2000; Brown and Aggleton 2001) for review see (Brown and Aggleton 2001). It is possible that the cortical coding of object recognition decays fast and is not sufficient for maintaining information about objects across longer retention intervals (e.g., 24 hr). Therefore, strong novel object preference after long (but not short) delays would require hippocampal object memory encoding. Future studies using acute intracranial injection of lidocaine may prove useful in dissociating the contributions of the perirhinal cortex and the hippocampus in the processes of object recognition, and the encoding and retention of object memory.

Alternative Hypotheses

It is possible that in the long delay experiment of the present study, the object memory impairment is due to a state-dependent effect, since lidocaine is only present during the sample session. However, since there were no effects of intra-hippocampal lidocaine treatment found on multiple noncognitive behavioral measures of performance,

such as velocity and latency to accumulate sample exploration, it is likely that there is not a profound difference in subjective state with intra-hippocampal lidocaine administration. In addition, during the test session in this experiment, no differences between treatment groups were found in velocity (t (19) = -0.933; $P > 0.05$), total distance moved (t (19) = -1.237; $P > 0.05$), or thigmotaxis (t (19) = 0.495; $P > 0.05$). Regardless, effects of state dependency will need to be ruled out in future studies with intra-hippocampal lidocaine administration either after the sample session or prior to both the sample and test sessions following a 24-hour retention interval.

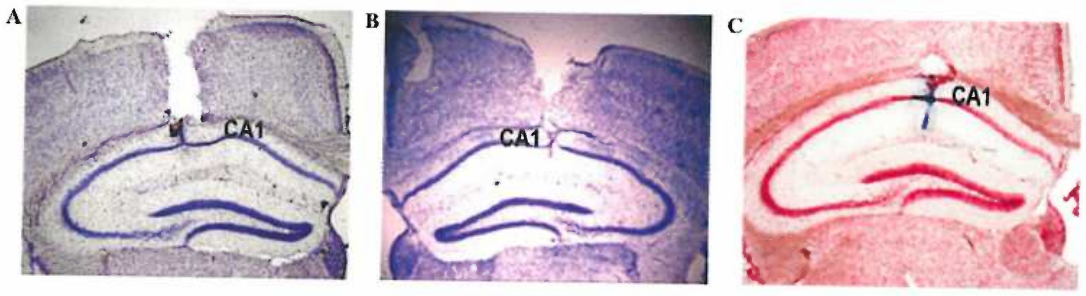
Another popular explanation for a hippocampal role in object memory is that hippocampal activity is required only for spatial components of the task. There have been a variety of studies that suggest that the hippocampus is necessary only for the spatial aspects of object recognition memory. The hippocampus has long been well characterized in spatial navigation and spatial memory (O'Keefe and Nadel 1978; Morris, Schenk et al. 1990), and other studies have demonstrated the involvement of the hippocampus in spatial object recognition tasks (Wan, Aggleton et al. 1999; Brown and Aggleton 2001; Mumby, Gaskin et al. 2002). For example, rats with radiofrequency lesions of the fornix (Ennaceur, Neave et al. 1997; Bussey, Duck et al. 2000; Warburton, Baird et al. 2000) and excitotoxic lesions of the hippocampus (Liu and Bilkey 2001; Mumby, Gaskin et al. 2002) are impaired in their ability to discriminate novel locations or novel contexts of familiar objects after a retention interval of 15 min or less. However, the present study was designed so that spatial or contextual cues were constant across all sessions. Mice were thoroughly habituated to the arena before training, so that the arena context was not drastically different during training and testing. In addition, objects were located in

opposite and symmetrical corners of the arena, and location of novel versus familiar object was counterbalanced. Furthermore, except for the retention interval, procedures of both experiments in the present study were identical, with equal spatial and contextual components. Therefore, findings from the present study demonstrate that the hippocampus is in fact involved in nonspatial memory (in this study, specifically in object memory encoding). More experiments will be needed to elucidate the specific role of the hippocampus in different object memory processes.

Table 2.1. *Intra-hippocampal lidocaine administration does not significantly alter performance measures of mice in the spontaneous object recognition task.*

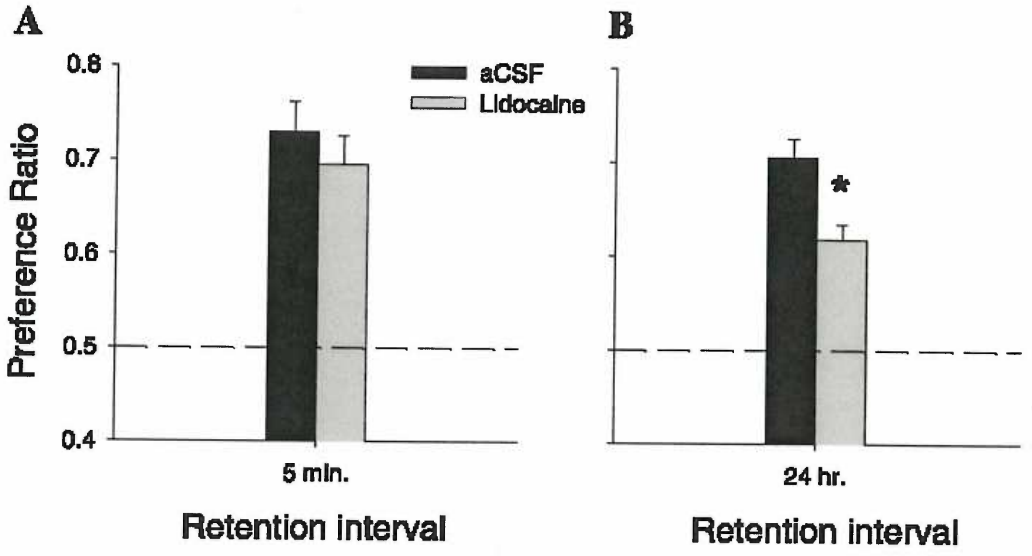
	Retention Interval			
	5 min		24 hr	
	aCSF	Lidocaine	aCSF	Lidocaine
Sample Session				
Latency to 38 sec. exploration (min)	5.89 ± 0.49	5.73 ± 0.44	5.95 ± 0.55	6.61 ± 0.76
Velocity (cm/sec)	5.57 ± 0.28	6.59 ± 0.47	5.99 ± 0.44	6.35 ± 0.69
Total distance Moved (m)	18.97 ± 1.70	21.64 ± 1.42	19.94 ± 1.20	23.30 ± 2.65
Thigmotaxis (% time in periphery)	89.29 ± 2.77	86.62 ± 1.34	87.10 ± 2.50	90.42 ± 2.01
Test Session				
Velocity (cm/sec)	5.20 ± 0.36	5.86 ± 0.43	6.82 ± 0.22	7.20 ± 0.37
Total distance Moved (m)	15.55 ± 1.06	17.45 ± 1.28	19.63 ± 0.76	21.25 ± 1.13
Thigmotaxis (% time in periphery)	89.12 ± 1.57	87.05 ± 2.46	88.66 ± 1.49	87.66 ± 1.21

Figure 2.1.



Photomicrographs of guide cannulae placement in dorsal CA1 of the hippocampus. Images from three mice are representative of cannulae placement seen in included subjects, with injection cites within 50 μm of the CA1 pyramidal cell layer. (A,B) Brain slices were stained with Cresyl violet. (C) This mouse received bilateral intra-hippocampal thionin injections (0.5 μl/side) prior to euthanasia, and brain slices were background stained with neutral red.

Figure 2.2.



Neural inactivation of the hippocampus prior to training impairs object memory after a 24 hour (but not a 5 min) retention interval. Mice administered aCSF or intra-hippocampal lidocaine (4%) prior to training in the spontaneous object recognition task showed novel object preference after a 5 min or 24 hour retention interval. (A) After a 5 min retention interval, lidocaine-treated mice exhibit similar novel object preference as aCSF-treated mice as measured by the preference ratio (novel object exploration / total object exploration during test session). (B) However, after a 24 hour retention interval, lidocaine-treated mice exhibit significantly weaker novel object preference than aCSF-treated mice. These data suggest that neural activity in the dorsal hippocampus is required for long-term object memory.

III. SK CHANNEL REGULATION OF OBJECT MEMORY ENCODING

Abstract

Small conductance Ca^{2+}-activated K^+ (SK) channels have been shown to regulate hippocampal cell excitability, synaptic plasticity, and spatial learning and memory. However, it remains unclear whether SK channels specifically modulate memory encoding or memory retention. In this study, we examined the role of SK channels in object recognition memory using apamin, a selective SK channel blocker. In Experiment 1, mice were treated systemically with apamin (0, 0.4 mg/kg) 30 min prior to extensive training, and object memory was tested after a 24- or 48-hour retention interval with no difference between treatment groups. Both apamin- and vehicle-treated groups exhibited significant novel object preference after a 24 hour retention interval, but not after a 48-hour retention interval. Therefore, systemic apamin does not affect object memory retention. In Experiment 2, mice were treated systemically with apamin (0, 0.4 mg/kg) 30 min prior to either limited (19 sec of sample object exploration) or extensive training (38 sec). After a 24 hour retention interval, apamin- and vehicle-treated mice allowed extensive sample object training exhibited similar novel object preference. However, when limited in training, apamin-treated mice exhibited significantly more novel object preference than control mice, indicating that systemic apamin enhances object memory encoding. In Experiment 3, apamin was administered directly into the dorsal hippocampus via bilateral hippocampal cannulation. Intra-hippocampal apamin (0, 1ng/side) was administered 5 min prior to limited training and object recognition memory was assessed. With limited training, mice administered intra-hippocampal apamin but not aCSF exhibited significant novel object preference during the first 2 min of the test session. Together, these data indicate that hippocampal SK channels specifically regulate

object memory encoding.

Introduction

The hippocampus is an essential structure for learning and memory in humans (Squire 1992) and rodents (Morris, Schenk et al. 1990; Eichenbaum 1999; Clark, Zola et al. 2000). Specifically, the hippocampus has been implicated in the encoding, consolidation, and retrieval (but not storage) of declarative memories (memories for facts, events, places). Humans with hippocampal lesions characteristically exhibit severe anterograde amnesia, but only temporally graded retrograde amnesia, suggesting that the location of memory storage resides outside of the medial temporal lobe (Squire 1992; Teng and Squire 1999). This phenomenon has also been observed in primate and rodent models of declarative memory (Winocur 1990; Zola-Morgan and Squire 1990). Understanding mechanisms of this memory system is of great importance because deficits in declarative memory are common in many neurological disease states, including senile dementia, Alzheimer's disease, and stroke.

Long-term potentiation (LTP) and long-term depression (LTD) are two forms of synaptic plasticity thought to underlie hippocampal memory formation (Bliss and Collingridge 1993; Malenka and Nicoll 1999). These activity-dependent long-term changes in synaptic strength are regulated by a variety of molecular substrates, including small conductance Ca^{2+}-activated K^+ (SK) channels. SK channels are activated by increases in intracellular calcium in a voltage-independent manner. SK channels have been shown to regulate cell excitability (Stocker, Krause et al. 1999; Cai, Liang et al. 2004) and plasticity in CA1 neurons (Behnisch and Reymann 1998; Stackman, Hammond et al. 2002; Kramar, Lin et al. 2004). In our working model of SK channel regulation of hippocampal function, we hypothesize that SK channels attenuate NMDA

receptor activation. At resting membrane potentials, Mg^{2+} ions block the pore of NMDA receptors, therefore NMDA receptor activation requires coincident membrane depolarization (to remove the Mg^{2+} block) and glutamate binding. Once activated, NMDA receptors pass Ca^{2+} ions into the postsynaptic cell, thereby activating SK channels (Shah and Haylett 2002). Activation of SK channels results in membrane hyperpolarization, which then reinstates the Mg^{2+} block of NMDA receptors, and attenuates further NMDA receptor activation. In support of this model, it has recently been shown that SK channels regulate the NMDA receptor-mediated component of the excitatory postsynaptic potential in CA1 neurons (Ngo-Anh, Bloodgood et al. 2005).

In addition to modulating hippocampal synaptic plasticity, SK channels also regulate hippocampal-dependent learning and memory (Ikonen, Schmidt et al. 1998; van der Staay, Fanelli et al. 1999; Stackman, Hammond et al. 2002). However, it remains unclear which hippocampal memory processes (encoding, retention, or retrieval) are modulated by SK channels. In the Morris water maze, apamin-treated mice require less training to learn the location of the hidden platform than control mice, suggesting that apamin specifically enhances memory encoding in this spatial, hippocampal-dependent task (Stackman, Hammond et al. 2002). However, in this experiment, spatial learning was assessed with probe tests administered after training, during which the platform is removed from the pool and the search behavior of the mouse is analyzed. Since apamin was still present during the probe tests in these mice, it is possible that apamin influences memory retrieval processes. This was also observed in another study, in which mice administered apamin only prior to a probe test exhibited improved search behavior for the hidden platform in the Morris water maze (van der Staay, Fanelli et al. 1999). A role for

apamin specifically in memory encoding has also been observed in rats. In an object recognition task, pre-training apamin enhances object recognition memory but this enhancement is not observed if apamin is administered after training or prior to testing (Deschaux, Bizot et al. 1997). However, in this study, the retention interval was short (1 hour), allowing for the possibility that apamin was present during the test session, thereby influencing memory retrieval.

To determine if SK channels specifically modulate object memory encoding in mice, we examined the effects of apamin (systemic and intra-hippocampal) on object memory processes using the spontaneous object recognition task. Lesion studies have demonstrated that this task is hippocampal-dependent (Clark, Zola et al. 2000; Hammond, Tull et al. 2004), and therefore may be useful as a rodent model of declarative memory. In addition, this task is beneficial for examining neurobiological mechanisms of memory because it relies upon a rodent's natural exploratory behavior, and does not require direct reinforcement. Many tasks that test rodent models of declarative memory rely on food restriction and reward, shock, or other aversive stimuli. In this task, mice explore and encode information about two identical objects during a sample session. After a given retention interval, mice are presented with one familiar and one novel object during a test session. If information about the sample objects was sufficiently encoded and maintained across the retention interval, then during the test session mice should explore the novel object more than the familiar object, since rodents have a natural preference for novelty (Ennaceur and Delacour 1988). To examine memory retention in this task, the length of the retention interval can be manipulated. Likewise,

memory encoding can be examined by manipulating the amount of training (in this task, the amount of sample object exploration).

To examine the role of SK channel function in object memory *retention*, in experiment I systemic apamin was administered to mice prior to the sample session and object memory was assessed after a 24- or 48-hour retention interval. To examine the role of SK channels in object memory *encoding*, in experiment II systemic apamin was administered prior to limited or extensive sample session training, and object memory was assessed after a 24-hour retention interval. Lastly, to examine the specific role of *hippocampal* SK channels in object memory encoding, in experiment III intra-hippocampal apamin was administered prior to limited sample session training and object memory was assessed after a 24 hour retention interval. Results from the present study indicate that systemic apamin administration enhances object memory encoding, but not retention.

Methods

General Methods:

Subjects: Naïve male C57BL/6J mice 6-8 weeks old (Jackson Labs, Bar Harbor, ME) were group housed and kept on a 12-hour light/dark cycle with food and water available continuously. All experiments were performed in accordance with the National Institute of Health Guidelines and the OHSU Institutional Animal Care and Use Committee.

Spontaneous Object Recognition Task: This task was administered as described previously in section II of this thesis (page 45). Briefly, Mice were familiarized to objects

during a sample session, in which two identical objects were placed in opposite corners of the arena, approximately 2 cm from the wall. Exploration was hand scored using the EthoVision system and was defined as time spent with the head oriented towards and within 2-3 cm of the object, and with the vibrissae moving. After a retention interval, mice were placed into the arena containing one sample (familiar) object and one novel object for a 5 min test session.

Data Analysis: All data are represented as mean ± SEM. In each experiment novel object preference is expressed as a *preference ratio*, which is calculated by dividing the amount of exploration of the novel object by the total amount of object exploration during the test session. Therefore, a preference ratio above 0.5 would indicate novel object preference, below 0.5 familiar object preference, or equal to 0.5 no preference (chance performance).

Experiment 1

Spontaneous Object Recognition Task: Mice received systemic apamin (0, 0.4 mg/kg, i.p.) 30 min prior to the sample session. During the sample session, mice were allowed 5 min to explore the two identical sample objects. After a 24-hour retention interval, mice were placed back into the arena for a 5 min test session and novel object preference was assessed. Four days later, these mice were retrained in the spontaneous object recognition task with a new object pair, and this time the test session was administered after a 48-hour retention interval. Prior to this study, both object pairs were tested with a separate group of mice to establish that they elicited equal exploration.

Data Analysis: Sample session average velocity, sample session total distance moved (TDM), and test session preference ratios were analyzed with 2-factor (retention interval X treatment) analyses of variance (ANOVAs). Independent samples Student's *t*-tests were used for post-hoc analysis where appropriate.

Experiment 2

Spontaneous Object Recognition Task: Mice received systemic apamin (0, 0.4 mg/kg) 30 min prior to the sample session. During the sample session, mice were allowed to explore sample objects until accumulating either 19 sec (limited training) or 38 sec (extensive training) of exploration on one of the sample objects. These time points were chosen based on preliminary findings that C57BL/6J mice typically accumulate 38 sec of object exploration in a 5 min sample session. A maximum of 10 min was allotted for each sample session.

Data Analysis: All data are represented as mean ± SEM. Sample velocity, and sample TDM were analyzed with 2-factor (sample exploration X treatment) ANOVAs. Latency to accumulate sample object exploration and test session preference ratios were analyzed for 19 or 38 sec exploration groups separately with planned comparison's *t*-tests, since we hypothesized that apamin would enhance novel object preference with limited (19 sec), but not extensive (38 sec) sample object exploration.

Experiment 3

Surgery: Fifty mice were implanted with bilateral guide cannulae positioned above the

CA1 region of the dorsal hippocampus. All surgeries were performed under aseptic conditions as described in section II of this thesis (page 44).

Intra-Hippocampal Infusions: Mice were administered bilateral intra-hippocampal infusions of 0.5 µl/side apamin (0, 1 ng) in aCSF (in mM: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 2.2 dextrose, 1.7 $CaCl_2$-$2H_20$, 35.9 $NaHCO_3$, pH 7.4) as previously described in section II of this thesis (page 47).

Spontaneous Object Recognition Task: Mice were administered intra-hippocampal apamin (0, 1 ng) 10 min prior to the sample session. Sample sessions were ended after each mouse accumulated 19 sec of exploration with one of the sample objects.

Data Analysis: For experiment 3, independent samples Student's *t*-tests were used for each analysis.

Histology: Histological analysis of cannulae placement was performed as described in section II of this thesis (page 47). Briefly, brains were sectioned coronally (40 µm), mounted onto gelatin-coated glass slides, and stained with Cresyl violet. Cannula placement was determined using a light microscope (Zeiss Axioplan, West Germany) and mice with infusion sites greater than 50 µm from dorsal CA1 were excluded from the behavioral analyses (n=12).

Results

Experiment 1: Systemic apamin administration does not affect object memory retention

To examine the effects of apamin on object memory retention, novel object preference was tested after a 24- or 48-hour retention interval in mice treated systemically with apamin (0.4 mg/kg; n=14) or vehicle (saline, n=12) 30 min prior to sample session training. No effect of apamin was found on average velocity ($F_{1, 22}$=4.15; p>0.05) or total distance moved (TDM; $F_{1, 22}$=2.98; p>0.05) during the sample session, indicating that systemic apamin did not severely disrupt basal motor activity (Table 2). No effect of apamin was found on novel object preference during the test session, as measured by test session preference ratios (Fig.3.1; $F_{1, 22}$=2.06; p>0.05), indicating that apamin does not alter object memory retention. There was a significant effect of retention interval on novel object preference ($F_{1, 22}$=20.30; p<0.001), indicating that object memory was less stable across a 48-hour interval than a 24-hour interval. In fact, post-hoc analyses revealed that both apamin- and vehicle-treated mice exhibited significant novel object preference after a 24-hour retention interval (t_{12}=7.05; p<0.001), but not after a 48-hour retention interval (t_{12}=0.81; p>0.05).

Experiment 2: Systemic apamin administration enhances object memory encoding

To examine the effects of apamin on object memory encoding, mice were treated systemically with apamin (0.4 mg/kg; n=20) or vehicle (saline; n=18) 30 min prior to either limited (19 sec) or extensive (38 sec) sample object training, and novel object preference was examined after a 24-hour retention interval. Apamin improved object

memory encoding in this task (Fig. 3.2). When limited to 19 sec of sample object exploration, apamin-treated mice exhibited significantly more novel object preference than vehicle-treated control mice (t_{17}= -2.17; p<0.05). However, when allowed extensive (38 sec) sample object exploration, apamin- and vehicle-treated mice exhibited similar novel object preference (t_{17}= -0.11; p>0.05). These data reveal that blockade of SK channels enhances object memory encoding, since less training is required for apamin-treated mice to demonstrate novel object preference after a 24-hour retention interval. Importantly, this effect was not due to differences in motivation to explore objects during the sample session, since no effect of apamin was found on the latency to accumulate 19 sec (t_{17}= -1.31; p>0.05) or 38 sec (t_{17}= 0.04; p>0.05) of sample object exploration (Table 1). In addition, apamin did not alter sensorimotor ability, since there was no effect of apamin on average velocity ($F_{1, 34}$=0.04; p>0.05) or TDM ($F_{1, 34}$=1.29; p>0.05) during the sample session (Table 2).

Experiment 3: Intra-hippocampal apamin administration enhances object memory encoding

Above, we have shown that systemic apamin administration enhances object memory encoding. To assess whether these behavioral effects of apamin are due specifically to the blockade of hippocampal SK channels, we examined object memory encoding in mice administered intra-hippocampal apamin (1 ng; n=18) or vehicle (aCSF; n=20) 5 min prior to limited (19 sec) sample object exploration. Representative photomicrographs of CA1 cannulae placement are illustrated in Fig. 3a. Intra-hippocampal apamin did not affect motivation to explore objects during the sample

session, since no effect of apamin was found on the latency to accumulate sample object exploration (Table 2; t_{36}=0.21; p>0.05). In addition, apamin did not alter motor activity during the sample session as measured by average velocity (t_{36}=0.83; p>0.05) or total distance moved (Table 2; t_{36}=0.88; p>0.05).

After a 24-hour retention interval, no differences were found between apamin- and vehicle-treated mice in preference ratios during the 5 min test session (t_{36}=0.14; p>0.05), indicating that intra-hippocampal apamin does not enhance object memory encoding (Fig. 3.3b). We also examined preference ratios during the first two minutes of the test session only (Fig.3.3c), since rats (Dix and Aggleton 1999) and mice (Stackman lab, unpublished observations) exhibit more robust object discrimination during the first two minutes. Over the 5 min test session, there is opportunity for the novel object to become familiar to the mouse. Therefore, object exploration during the first two minutes may be a more accurate reflection of object preference. When examining preference ratios during the first 2 minutes only of the test session (24 hours after limited (19 sec) sample object exploration), apamin-treated mice exhibited significant novel object preference (t_{17}=5.96; p<0.001), while vehicle-treated mice did not (t_{19}=1.59; p>0.05). These data support the hypothesis that apamin influences object memory encoding through blockade of hippocampal SK channels.

Discussion

Our findings indicate that SK channels regulate hippocampal–dependent memory encoding, but not memory retention, in the object recognition paradigm. Systemic apamin- and vehicle-treated mice remembered object information equally well across a 24-hour retention interval, and did not remember object information across a 48-hour interval. Also, apamin-treated mice required less training (sample object exploration) than controls to remember object information across a 24-hour retention interval. We conclude that SK channels specifically regulate object memory *encoding*, since (1) apamin was only administered prior to sample session training (and was not present during memory retrieval) and (2) apamin did not alter novel object preference with extensive training. If SK channels were involved in memory retention processes, one might expect apamin to enhance novel object preference regardless of the amount of training during the sample session. In this way, our modified version of the object recognition task permits a specific examination of memory encoding.

We have also found in the present study that SK channels *in the hippocampus* may enhance object memory encoding, since apamin- but not vehicle-treated mice exhibited novel object preference in the first 2 minutes of the test session. However, we cannot conclude from these data that apamin's effect is solely through hippocampal SK channel blockade for two reasons. First, there was not a significant difference between apamin- and vehicle-treated mice in novel object preference during either the full 5 min test session, or for the first 2 minutes of the test session. Second, the magnitude of novel object preference was enhanced to a greater degree with systemic apamin administration (experiment 2), than with intra-hippocampal apamin administration (experiment 3). It is

possible that the minimal effect of intra-hippocampal apamin in this experiment is due to an insufficient dose. This is the first study to use intra-hippocampal apamin injections. In preliminary tests in our lab, we also examined the effects of a higher dose of intra-hippocampal apamin (5 ng/side), but unfortunately, this dose caused behavioral seizures in the mice. Therefore, the 1 ng/side dose of intra-hippocampal apamin in the present study may have had a less robust effect on object memory encoding than systemic apamin because this dose is insufficient for complete hippocampal SK channel blockade. Likewise, it is possible that hippocampal SK channel blockade was insufficient because apamin was only administered to the dorsal portion of CA1. Hippocampal inactivation studies from section II of this thesis have shown that lidocaine administered into the dorsal CA1 region with this injection procedure sufficiently impairs memory in the object recognition task (Hammond, Tull et al. 2004). However, it is possible that a larger population of hippocampal SK channels must be blocked by apamin to effectively enhance memory encoding, and this injection procedure did not distribute apamin to a large enough volume of the hippocampus. A final possibility is that SK channels in other brain regions additionally regulate object memory encoding. SK channels are located throughout structures afferent (entorhinal cortex, locus ceruleus, medial septum, raphe) and efferent (subicular complex, anterior thalamus, amygdala, entorhinal cortex) to the hippocampus (Stocker and Pedarzani 2000). Together, these data suggest a role for hippocampal SK channels in object memory encoding, although it is not clear whether SK channels in other brain regions also contribute to memory encoding.

Mechanistically, it seems likely that hippocampal SK channels are responsible for the regulation of object memory encoding. Although SK channels are expressed

throughout the central nervous system, their expression levels are quite high in cortical and limbic structures (Stocker and Pedarzani 2000). In addition, SK channels are likely to influence cell excitability and plasticity through their regulation of N-methyl-D-aspartate (NMDA) receptor activation in the hippocampus (Ngo-Anh, Bloodgood et al. 2005). NMDA receptors are required for the induction of synaptic plasticity in the CA1 region of the hippocampus (Malenka and Nicoll 1999), and many studies have shown the importance of NMDA receptor function in hippocampal learning and memory (Tsien, Huerta et al. 1996; Tang, Shimizu et al. 1999), including object recognition memory (Puma, Baudoin et al. 1998; Puma and Bizot 1998; Baker and Kim 2002).

In our model, we propose that SK channels attenuate NMDA receptor activation, and therefore limit hippocampal synaptic plasticity (and hippocampal learning and memory). Therefore, blocking SK channels with apamin would create a form of disinhibition resulting in enhanced NMDA receptor activation, synaptic plasticity, and ultimately learning and memory. Overall, results from the present study support our model that SK channels play an important role in regulating hippocampal function. It may seem counter-intuitive that SK channels are basally suppressing hippocampal synaptic plasticity, learning, and memory; after all, how would this mechanism benefit the organism? However, by attenuating these processes, SK channels may serve to maintain the homeostasis of synaptic plasticity in the hippocampus. For example, SK channels may be involved in ensuring synapse specificity during information processing, by limiting NMDA receptor activation to only those synapses which receive large enough postsynaptic depolarization. Together, findings from the present study further implicate

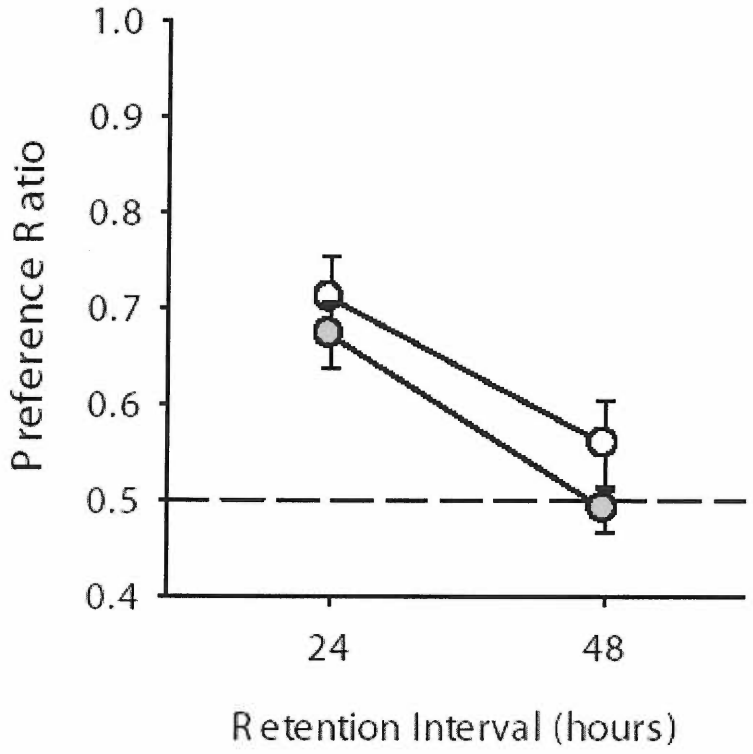
SK channels in the regulation of hippocampal learning and memory, and demonstrate that SK channels play a specific role in hippocampal memory encoding processes.

Table 3.1. *Apamin does not alter non-cognitive performance measures.*

	N	Sample Session Average Velocity (cm/sec)	Sample Session Total Distance Moved (cm)	Latency to Accumulate Sample Object Exploration (min) for:	
				Limited (19s)	Extensive (38s)
Experiment 1:					
Vehicle (saline, i.p.)	12	6.12 ± 0.17	1774.99 ± 54.69	n/a	n/a
Apamin (0.4 mg/kg, i.p.)	14	5.64 ± 0.16	1656.79 ± 45.39	n/a	n/a
Experiment 2:					
Vehicle (saline, i.p.)	18	5.81 ± 0.34	1526.26 ± 175.61	3.14 ± 0.68	6.56 ± 0.66
Apamin (0.4 mg/kg, i.p.)	20	5.73 ± 0.26	1799.73 ± 195.57	4.81 ± 1.03	6.52 ± 0.75
Experiment 3:					
Vehicle (aCSF, ihpc)	20	6.83 ± 0.41	1396.37 ± 142.91	3.73 ± 0.46	n/a
Apamin (1ng/side, ihpc)	18	7.20 ± 0.43	1470.74 ± 184.12	3.59 ± 0.47	n/a

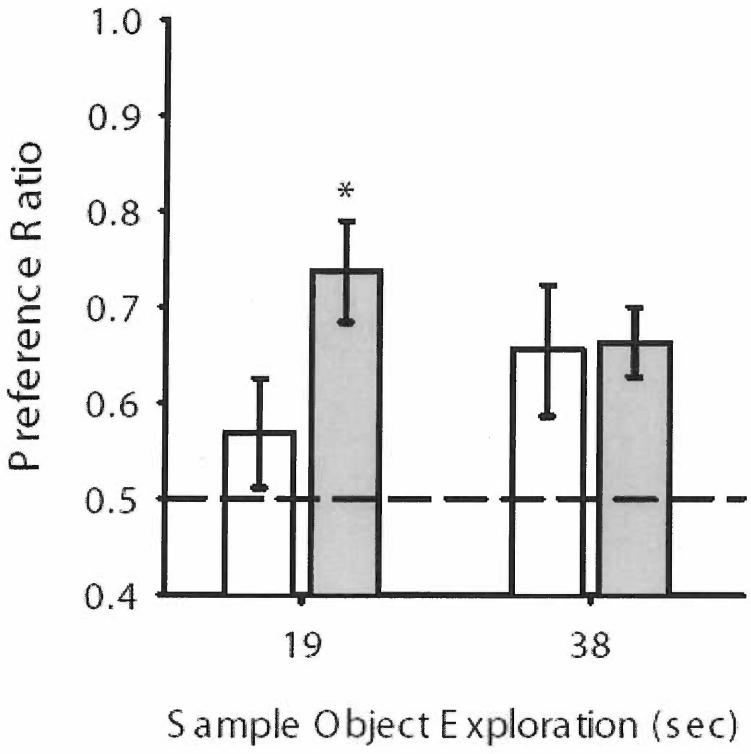
Data are represented as Mean ± SEM.
For each experiment, apamin was administered prior to the sample session.

Figure 3.1.



Systemic apamin does not affect object memory retention. Mice were treated with systemic apamin (0.4 mg/kg, gray circle) or vehicle (open circle) 30 min prior to sample session training. Preference ratios were calculated as seconds of novel object exploration/seconds of total object exploration during the 5 min test session. After a 24-hour retention interval, apamin- and vehicle-treated mice exhibited similar novel object preference ($p>0.05$). After a 48-hour retention interval, neither apamin- or vehicle-treated mice exhibited significant novel object preference ($p>0.05$), indicating that object memory retention has declined similarly in apamin- and vehicle-treated mice.

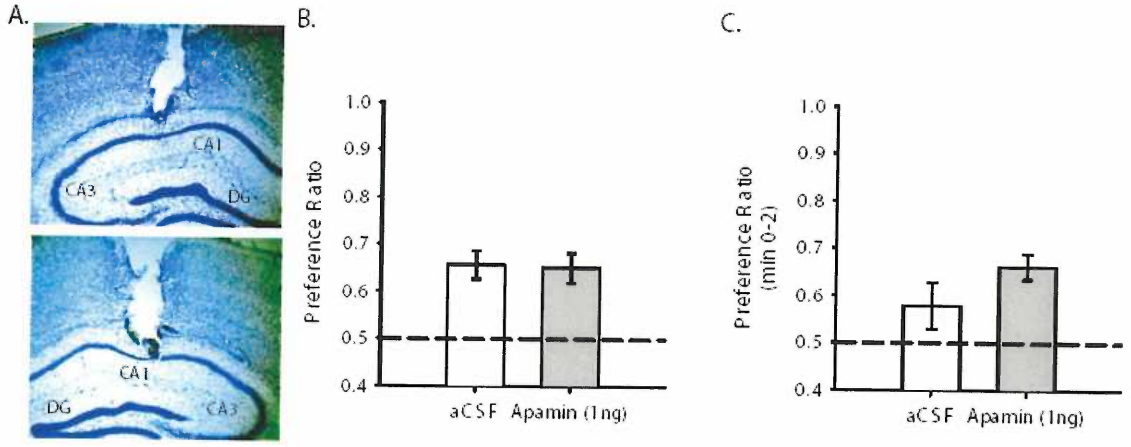
Figure 3.2.



Systemic apamin enhances object memory encoding. In this experiment, the object recognition task was modified to examine the effects of apamin on object memory encoding. Mice were treated with systemic apamin (0.4 mg/kg, gray bar) or vehicle (open bar) 30 min prior to limited (19 sec) or extensive (38 sec) sample session training. After a 24-hour retention interval, preference ratios during the 5 min test session were analyzed with planned comparisons Student's *t* tests. When sample session training was limited (19 sec), apamin-treated mice exhibited significantly greater novel object preference than vehicle-treated mice ($p<0.05$). However, with extensive training (38 sec), both apamin- and vehicle-treated groups exhibited similar novel object preference ($p>0.05$). These data indicate that systemic apamin enhances object memory encoding, since less training was

necessary for apamin-treated mice to exhibit novel object preference after a 24-hour retention interval. * indicates $p<0.05$.

Figure 3.3.



Intra-hippocampal apamin may enhance object memory encoding. Mice received bilateral intra-hippocampal infusions of apamin (1ng/side) or vehicle 5 min prior to limited (19 sec) sample session training. (a) Representative photomicrographs show cannulae placement just above the CA1 region of the dorsal hippocampus. After a 24-hour retention interval, preference ratios during the 5 min test session or the first 2 min only of the test session were analyzed with independent sample Student's *t* tests. (b) When examining the full 5 min test session, apamin- and vehicle-treated mice exhibited similar novel object preference ($p>0.05$). (c) Because novel object preference may be more accurately reflected during initial exposure to objects in the test session, we also examined novel object preference within the first two minutes of the test session. During the first two minutes of the test session, apamin-treated mice exhibited significant novel object preference ($p<0.001$), while vehicle-treated mice did not ($p>0.05$). These data support the notion that hippocampal SK channels are involved in the regulation of object memory encoding.

IV. SK2 CHANNEL OVEREXPRESSION IMPAIRS HIPPOCAMPAL LEARNING, MEMORY, AND SYNAPTIC PLASTICITY

Abstract

Apamin-sensitive small-conductance Ca^{2+}-activated K^+ channels (SK channels) modulate neuronal excitability in CA1 neurons. Blocking all SK channel subtypes with apamin facilitates the induction of hippocampal synaptic plasticity and enhances hippocampal learning. In CA1 dendrites, SK channels are activated by Ca^{2+} through NMDA receptors (NMDARs), and restrict glutamate-mediated excitatory postsynaptic potentials (EPSPs). Studies of SK channel knockout mice reveal that of the three apamin-sensitive SK channel subunits (SK1-3), only SK2 subunits are necessary for the apamin-sensitive currents in CA1 hippocampal neurons. To determine the specific influence of SK2 channels on hippocampal synaptic plasticity, learning and memory, we used gene targeting through homologous recombination in ES cells to generate transgenic mice that overexpress SK2 subunits by 10-fold (SK2+/T). In these mice, the apamin-sensitive current in CA1 neurons was increased by approximately 4-fold, relative to wild type (WT) littermates. In addition, SK2 overexpression augmented the suppression of synaptically-evoked EPSPs; treatment with the SK channel blocker apamin increased the EPSP amplitude of CA1 neurons by twice that of CA1 neurons from WT mice. The amplitude of synaptically evoked EPSPs recorded from SK2+/T CA1 neurons increased twice as much in response to SK channel blockade relative to EPSPs recorded from WT CA1 neurons. Consistent with this, SK2 overexpression reduced long-term potentiation after high frequency stimulation compared to WT littermates, and severely impaired learning in both hippocampus- and amygdala-dependent tasks. We conclude that SK2 channels regulate hippocampal synaptic plasticity and play a critical role in modulating mechanisms of learning and memory.

Introduction

The hippocampus is an essential brain structure for declarative memory formation which involves memories that can be consciously recollected (Eichenbaum 2000). In rodent models of declarative memory, lesion studies have demonstrated that the hippocampus is involved in similar memory processes, including learning and remembering complex associations between stimuli (Morris, Garrud et al. 1982; Squire 1992; Eichenbaum 1999). Long-term potentiation (LTP) and long-term depression (LTD) are thought to represent cellular mechanisms of memory formation that involve activity-dependent long-term changes in synaptic strength and require N-methyl-D-aspartate receptor (NMDAR) activation (Malenka and Nicoll 1999).

Small-conductance Ca^{2+}-activated K^+ channels (SK channels) regulate membrane excitability in CA1 neurons and modulate hippocampal synaptic plasticity and learning (Stackman, Hammond et al. 2002). SK channels are voltage-independent and are activated by sub-micromolar concentrations of intracellular Ca^{2+} ions. Apamin, a selective SK channel antagonist, enhances the excitability of CA1 neurons (Stocker, Krause et al. 1999; Cai, Liang et al. 2004). In addition, SK channels regulate synaptically-evoked excitatory postsynaptic potentials (EPSPs). During an EPSP, dendritic SK channels are activated by increases in spine $[Ca^{2+}]_I$, resulting in repolarization of the spine membrane and reinstatement of the Mg^{2+} block of NMDARs (Faber, Delaney et al. 2005; Ngo-Anh, Bloodgood et al. 2005). This SK channel-mediated feedback loop provides one mechanism by which SK channels could regulate hippocampal synaptic plasticity. Consistent with this hypothesis, blockade of SK channels by apamin decreases the threshold for the induction of synaptic plasticity in

hippocampal slices (Stackman, Hammond et al. 2002; Kramar, Lin et al. 2004), and systemically administered apamin enhances hippocampal memory encoding (Stackman, Hammond et al. 2002).

There are three SK channel subtypes (SK1-3) expressed in mammalian brain (Kohler, Hirschberg et al. 1996). Although all three SK channel subtypes are expressed in the rodent hippocampus, SK1 and SK2 channels are present in layers CA1 and CA3 in significantly higher densities than SK3 channels (Stocker and Pedarzani 2000; Sailer, Hu et al. 2002; Sailer, Kaufmann et al. 2004). It is unclear from the current literature which specific SK channel subunit(s) contribute to the modulation of hippocampal plasticity and behavior. Transgenic mice, engineered to overexpress or lack one of the three SK channel subtypes, hold great promise for dissecting the unique contributions of each SK channel subtype to specific behavioral and physiological endpoints. For example, apamin-sensitive currents are absent in CA1 neurons from transgenic mice that lack SK2 subunits (Bond, Herson et al. 2004). Therefore, we hypothesize that SK2 channels underlie the apamin-mediated modulation of synaptic plasticity in CA1 neurons and hippocampal-dependent learning. To test this hypothesis, we examined subthreshold EPSPs in CA1 neurons, hippocampal synaptic plasticity and learning and memory in a transgenic mouse that overexpresses SK2 channels (SK2+/T). The apamin-sensitive current recorded from hippocampal slices in SK2+/T mice was 4-fold larger than in WT mice. Consistent with our previous study in which blockade of SK channels enhanced learning, overexpression of SK2 channels impaired hippocampal learning and memory and attenuated subthreshold EPSPs and synaptic plasticity in hippocampal slices. These data support our hypothesis that SK2 channels modulate hippocampal synaptic plasticity and learning.

Methods

Subjects

The Oregon Health and Science University IACUC approved all procedures. All mice were group housed, and kept on a 12-hour light/dark cycle with food and water available *ad libitum*. Mice were weaned at 3 weeks and genotyped. The experimenter was blind to genotype for all behavioral and electrophysiological experiments. Transgenic mouse lines were backcrossed greater than 6 generations onto the C57BL/6J background. For behavioral studies, heterozygous (SK2+/T) mice and WT littermates were used.

Transgenic mouse production[22]

A 7 kb NotI-SpeI fragment positive for hybridization with mSK2 N-terminal coding sequences was isolated from a lambda 129/Sv genomic library and cloned into Bluescript as a basis for the targeting construct. Subsequent sequence analysis of overlapping clones revealed the NotI site to be vector-derived. The tetracycline regulatory cassette (Bond, Sprengel et al. 2000) was inserted 40 nt 5' of the initiator MET by homologous recombination in yeast. The resulting targeting construct was electroporated into ES cells and G418-resistant colonies were analyzed for appropriate recombination by genomic DNA PCR and Southern blots. Two correctly targeted clones were injected into C57BL/6J blastocysts. One chimera gave germline transmission of the tTA allele. SK2 tTA mice were bred to a CRE deleter mouse (Bond, Sprengel et al. 2000), resulting in excision of the neomycin coding and URA gene sequences from the allele. The resulting line of mice, mSK2 $tTA_{\Delta neo}$, were maintained as heterozygotes after backcrossing into the C57BL/6J background, with breedings consisting of +/T males crossed with WT females.

[22] Transgenic mice were engineered by Dr. Chris Bond, Vollum Institute, Portland, OR.

Western Blot[23]

Membrane proteins were prepared from SK2+/T or WT mice as described previously (Strassmaier, Bond et al. 2005). Whole brains were homogenized in ice cold HS (in mM: 320 sucrose, 10 Hepes, 1 EGTA) with Sigma mammalian protease inhibitor cocktail. Protein content was determined using the BCA method (Pierce, Rockford, IL). Membrane proteins were mixed in SDS-PAGE loading buffer and separated on an SDS-PAGE gel. Membrane proteins were then transferred onto PVDF membranes and anti-SK2-C primary antibody (Bond, Herson et al. 2004) was diluted to 2 μg/ml in 3% powdered milk in PBS. HRP-conjugated secondary antibody was diluted 1:10,000 in PBS with 0.1% Tween-20, and blots were visualized with SuperSignal West Pico (Pierce, Rockford, IL).

Real-time PCR[24]

Whole-brain RNA was isolated with Tri-reagent, and total RNA was reverse-transcribed by Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) as reported previously (Bond, Herson et al. 2004). Expression levels of real-time PCRs were determined by comparison to 18S ribosomal RNA (rRNA) and were performed in triplicate. The amplicon for 18S was 76 bp (primers: CCGCAGCTAGGAATAATGGA, CCCTCTTAATCATGGCCTCA); for SK1, 118 bp (primers: GCTCTTTTGCTCTGAAATGCC, CAGTCGTCGGCACCATT GTCC); for SK2, 151 bp (primers: GTCGCTGTATTCTTTAGCTCTG, ACGCTCATAAG TCATGGC); for

[23] Western blot analysis was performed by Dr. Timothy Strassmaier, OHSU, Portland, OR.

[24] RT-PCR was performed by Dr. Chris Bond, Vollum Institute, Portland, OR.

SK3, 148 bp (primers: GCTCTGATTTTTGGGATGTTTG, CGATGATCA AACCAAGCAGGATGA). The threshold cycle (Ct) indicates the fractional cycle number at which the amount of amplified target reaches a fixed threshold. The reaction master mix, consisting of 1X buffer, Mg (C_f = 4 mM), deoxyribonucleotide triphosphates (C_f = 200 μM), Platinum taq polymerase (Invitrogen; 0.6 U/20 μl reaction), and SYBR Green (Molecular Probes, Eugene, OR; 0.5X recommended concentration of the manufacturer), was aliquoted, the cDNA substrates added, and then further aliquoted and primers added (C_f = 200 nM). Reactions were then split into triplicates for amplification in an MJ Research (Watertown, MA) Opticon DNA Engine with cycling parameters 95°C one time for 2 min, 95°C for 30 sec/64°C for 45 sec, with fluorescence read at 78°C for 40 cycles. A melting curve and gel electrophoresis analysis verified that a single product was amplified in all reactions. For each run, the relative mRNA level was determined by the expression $2^{-\Delta\Delta Ct}$ (ΔCt (SK_{Ct}-$18S_{Ct}$) within each genotype, $\Delta\Delta Ct$ ($\Delta Ct_{\text{SK transgene}}$- $\Delta Ct_{\text{wild type}}$) (ABI Prism 7700 Sequence Detection System, user bulletin 2; Applied Biosystems, Foster City, CA). The mean and s.e.m. of the value $2^{-\Delta\Delta Ct}$ were plotted for each SK mRNA across all runs.

Electrophysiological recordings:

Hippocampal slices were prepared from 3-5 week old SK2+/T or WT littermate mice. Mice were anesthetized with isoflurane, decapitated, and brains were immediately removed and placed into ice-cold cutting solution (in mM: 110 sucrose, 60 NaCl, 2.5 KCl, 28 $NaHCO_3$, 1.25 NaH_2PO4, 0.5 $CaCl_2$, 7 $MgCl_2$, 5 glucose, 0.6 ascorbate and equilibrated in 95% O_2 and 5% CO_2). Hippocampi were removed from the brain, placed

onto a 4% agar block, and transferred to the slicing chamber. Transverse slices (400 μM) were cut with a Vibratome (Leica VT 1000S; Leica, Nussloch, Germany) and transferred to an incubation chamber containing artificial CSF (ACSF, in mM: 125 NaCl, 2.5 KCl, 21.4 $NaHCO_3$, 1.25 NaH_2PO4, 2 $CaCl_2$, 1 $MgCl_2$, 11.1 glucose and equilibrated in 95% O_2 and 5% CO_2) maintained at 35°C for 30 min, then at room temperature for at least 90 min.

Whole cell recordings: CA1 neurons were visualized using a microscope with infrared/differential interference contrast optics (Leica DMLFS) and a CCD Camera (Sony, Tokyo, Japan). Recording pipettes were pulled from TW150F-4 thin-wall borosilicate glass (World Precision Instruments, Sarasota, FL). Pipettes had resistances of 1.5-3 MΩ and were filled with an intracellular solution containing (in mM): 140 $KMeSO_4$, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 2 Mg-ATP, 0.4 Na_2-GTP, and 20 μM EGTA, pH 7.3 (290 mOsm). Whole cell recordings were performed at room temperature (22-23°C) and during recordings each slice was continuously perfused with ACSF equilibrated to 95% O_2 / 5% CO_2. Whole-cell patch-clamp currents were acquired with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA), digitized using an ITC-16 converter (InstruTech, Greatneck, NY), and recorded onto a computer using Pulse software (Heka Elektronik, Lambrecht, Germany).

For measurement of the IAHP, CA1 pyramidal neurons were voltage clamped at –55 mV and tail currents were evoked with a depolarizing command to +20mV for 100ms, followed by a return to –55mV for 10-20s. After a stable baseline was established, apamin (100 nM, Calbiochem, La Jolla, CA) was applied to the bath, and the apamin-sensitive current was obtained by digital subtraction. Peak amplitudes of the apamin-

sensitive current were measured at 100 msec after the return to –55 mV. Access resistance was compensated at 80%. Current recordings were filtered at 1 kHz and digitized at a sampling frequency of 1 kHz; the records were filtered further off-line with a 300 Hz Gaussian filter (for one pass). Data were analyzed with IGOR Pro software (Wavemetrics, Lake Oswego, OR), and are presented as mean ± s.e.m.

For measurement of EPSPs[25], synaptic potentials were recorded in whole-cell current-clamp mode. A bipolar tungsten electrode (FHC) was used to stimulate presynaptic axons in stratum radiatum. Picrotoxin (0.1 mM) was added 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 100-μs current injections that were approximately one-third 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 approximately one-quarter of threshold. Recordings were made using an Axon 200A amplifier (Axon Instruments) interfaced to a Macintosh G4 with an ITC-16 computer interface (Instrutech Corp). Data were filtered at 5 kHz and collected at a sample frequency of 20 kHz using Pulse (Heka Elektronik). All recordings used cells with a resting membrane potential less than -60mV that did not change by more than 2 mV during an experiment and with a stable input resistance that did not change by more than 5%.

Field Recordings: Schaffer collaterals were stimulated with a bipolar tungsten electrode (FHC Bowdoinham, ME) placed into area CA3. A recording pipette (2-4 MΩ) filled with ACSF was placed into stratum radiatum of area CA1. Field excitatory

[25] EPSP recordings were performed by Jennifer Thu Ngo-Anh, Vollum Institute, Portland, OR

postsynaptic potentials (fEPSPs) were recorded at 30 ± 1°C. Following electrode placement, baseline synaptic transmission was monitored at 0.05 Hz, and a 10 min stable baseline was acquired prior to tetanization. In LTD experiments, 1 hour after 1 Hz stimulation, slices were tetanized with three 100 Hz tetani (100 pulses) at 0.1 Hz to ensure that bidirectional plasticity remained, and that LTD was not due to cell death. Data were digitized (10 kHz sampling rate) with a National Instruments AD interface and analyzed using IGOR (Wavemetrics, Lake Oswego, OR) on a Macintosh G3 computer (Apple, Cupertino, CA). The maximal initial slope of the field EPSP was measured to monitor the strength of synaptic transmission, minimizing contamination by voltage-dependent events. Summary graphs were obtained by normalizing each experiment according to the average value of all points on the 10 min baseline. All points were aligned with respect to the start of the (LTP or LTD) induction protocol, and each experiment was divided into 1 min bins and then averaged across experiments. The amount of potentiation or depression of the synaptic response was measured 30-40 min after conditioning.

Behavioral Tasks

For all behavioral procedures, 8-10 week old male mice were handled and weighed at least 2 days prior to the onset of the experiment.

Morris water maze: Naïve male SK2+/T (n = 14) and WT littermate (n = 17) mice were used in the Morris water maze paradigm. The pool was constructed of white polyethylene (60 cm high, 109 cm diameter) and pool water (22 - 24°C) was clouded with the addition of nontoxic white Tempra paint. Mouse behavior was recorded with a

video camera interfaced with the EthoVision 3.0 tracking system (Noldus, Leesburg, VA), permitting the acquisition of multiple behavioral parameters. Mice received 2 days of nonspatial training (1 trial/day) to acclimate to the pool and submerged platform, 2 days of visible platform training (6 trials/day) to examine sensorimotor function and striatal-dependent learning (McDonald and White 1994), and 11 days of hidden platform training (4 trials/day) to examine hippocampal-dependent spatial learning and memory (Morris, Garrud et al. 1982). During nonspatial training, a clear Plexiglas platform (13 cm dia) was located in the center of the pool, submerged 1 cm below the water surface, and uniform black curtains were drawn around the pool to block the animal's view of extra-maze visible cues. Each mouse was placed onto the platform for 60 s, then released into the pool (adjacent to platform, N, S, E and W) four times to practice swimming and climbing onto the platform. During visible platform training, the curtains were opened to allow view of the extra-maze cues and a black Plexiglas platform (13 cm dia) with a protruding flag was located just above the water's surface. Platform location and start site were randomized each trial. During hidden platform training, the platform was located in a fixed position in the center of the NE quadrant of the pool. Large visible cues were present around the pool. Start points around the edge of the pool (N, S, E and W) were randomized each day. Each mouse was allowed 60 s to reach the hidden platform, after which it was placed onto the platform. Mice remained on the platform for 30 s to view spatial cues, and then were placed into a holding cage for a 45 s inter-trial interval. To examine spatial memory retention after training, mice received a probe test 24 hr after the last training trial during which the platform was removed from the pool and mice were allowed to search for the platform for 60 s. A search ratio was calculated from probe test

behavior for each mouse as the number of crossings into a 23.8 cm diameter circular zone around the platform location, divided by the total number of crossings into four equivalent zones (from each pool quadrant N, S, E and W).

Fear conditioning: Contextual and cued fear conditioning were used to further assess the effects of SK2 overexpression on learning and memory. Learning in both contextual and cued fear conditioning tasks requires an intact amygdala, while contextual conditioning additionally requires hippocampal function (LeDoux 1993). Contextual conditioning involves a learned association between an aversive unconditioned stimulus (e.g., foot shock) and the environment in which it is presented (Kim and Fanselow 1992; Phillips and LeDoux 1992; Logue, Paylor et al. 1997). This learned association is observed as an increase in freezing behavior in response to the training context (Kim and Fanselow 1992). In contrast, cued fear conditioning involves the learned association between a discrete cue (e.g., tone) and the foot shock, with subsequent freezing to the cue alone, and this learning does not require an intact hippocampus (LeDoux 1993). In the present study, conditioned fear was examined in three replications, and data from these replications was combined since no effect of replication was found on any measure ($P > 0.05$). On the first day, naïve SK2+/T (total n = 42) and WT littermate (total n = 29) mice received a context pre-exposure session, in which each mouse was allowed to explore one of four automated activity chambers (Freeze Monitor, 20 x 25 x 12.5 cm, San Diego Instruments, San Diego, CA) for 5 min. Each activity chamber was located inside a sound-attenuating box containing a small fan for white noise and a house light. Activity chambers contained removable steel shock grid floors and clear Plexiglas ceiling and walls. The next day, each mouse was returned to the same chamber for conditioning, in

which three 30-s tones (68 dB, 30 Hz) were presented with a co-terminating 1 s foot shock (0.5 mA). These tone-shock pairings began after a 60 s delay, and were separated by a 180 s inter-stimulus interval. Chambers were cleaned with Liquinox detergent after each context pre-exposure and conditioning trial. To examine memory for contextual fear, each mouse was returned to the same chamber after a 24 hr retention interval. No shock or tone was presented during this 5 min context test. To examine cued fear conditioning, a tone test was conducted ~2 hr after the context test, in which each mouse was placed into a novel chamber that differed in location, odor (cleaned with 70% ethanol), sound (no fan), texture (smooth Plexiglas floor) and appearance (visual cues were added to chamber ceilings, floors, and walls; a Plexiglas divider diagonally bisected each chamber). During the tone test, the 30 s tone was presented twice after a 60 s delay and separated by a 180 s inter-stimulus interval. Since mice express conditioned fear as a freezing response, percent freezing was measured for each session.

Acoustic startle response and shock sensitivity were examined in separate cohorts of male SK2+/T and WT littermate mice to ensure that SK2 overexpression did not impair ability to hear and/or respond to the tone stimulus, or affect unconditioned responses of the mice to foot shock. Acoustic startle response to a 60 ms, 10 kHz tone was recorded with the Coulbourn Instruments (Allentown, PA) acoustic startle response system. Strain gauge transducers coupled to the startle platform were used to detect the jumping response. Tones varied in intensity (0, 60, 80,100 dB) and were presented pseudo-randomly to male SK2+/T (n = 6) and WT littermate (n = 7) mice, 40 trials/day for 2 days, with varying inter-trial intervals (15-55 s). The % change in peak amplitude of responses to 60, 80, or 100 dB (relative to 0 dB) measured on days 1 and 2 were averaged

for each mouse. To examine shock sensitivity thresholds, male SK2+/T (n = 6) and WT littermate (n = 6) mice were placed into the conditioning chambers for 60 s and then exposed to 0.5 s shocks every 120 s in order of increasing intensity (0.05, 0.25, 0.5, 0.63, 0.75 mA). The magnitude of shock (in mA) required to elicit a response (vocalization or running) was determined for each mouse.

Data Analysis

All data are represented as mean ± s.e.m. with significance set at $P > 0.05$. Real-time PCR was analyzed with a one factor analysis of variance (ANOVA). For electrophysiological recordings, field potential input-output relationships were analyzed with a 3-factor ANOVA, with genotype, fiber volley amplitude, and genotype X fiber volley amplitude as predictors of field EPSP slope. Paired pulse facilitation ratio data were analyzed with repeated measures ANOVA, and all other electrophysiological experiments were analyzed with two-tailed, independent samples Student's t-tests. For the Morris water maze, repeated measures ANOVA were used to assess behavior during visible and hidden platform training, analysis of covariance (ANCOVA) was used to examine differences during visible platform training, and two-tailed, independent samples Student's t-tests were used for all other comparisons. For fear conditioning and acoustic startle experiments, repeated measures ANOVAs were used, with post-hoc two-tailed Student's t-tests where appropriate. Student's t-tests were used to compare shock sensitivity threshold measures.

Results

SK2 Overexpressing mice

Transgenic mice overexpressing SK2 channels were developed via insertion of a tetracycline-activated gene switch into the SK2 locus (Bond, Sprengel et al. 2000). Compared to WT littermate mice, in the absence of doxycycline the SK2 protein and SK2 mRNA are overexpressed in heterozygotes (SK2+/T) approximately 10-fold and 4.5-fold as shown by western blot and quantitative PCR, respectively (Fig. 4.1a, b; $P < 0.001$). In addition real-time PCR revealed that the SK1 and SK3 mRNA levels were unchanged in brains from SK2+/T mice, relative to WT mice (Fig. 4.1b).

We have previously shown that SK2 channels are necessary for apamin-sensitive currents in CA1 hippocampal neurons (Bond, Herson et al. 2004). Tail currents following 100 ms depolarizations to 20 mV were recorded from CA1 neurons in the whole cell configuration. The addition of apamin to slices from SK2+/T mice revealed a 4-fold increase in the amplitude of the apamin-sensitive component of the IAHP current recorded 100 ms after repolarization (n = 7; 359.55 ± 48.59 pA) relative to WT (n = 13; 87.03 ± 7.34 pA; Fig. 4.2a-c; $P < 0.001$). SK2 overexpression did not alter the current recorded after 1 sec (IsAHP), the decay kinetics of the apamin-sensitive current (WT: 325.38 ± 39.05 ms, SK2+/T: 356.86 ± 63.13 ms; Fig. 4.2d), or the input resistance at -55 mV (WT: 276.82 ± 18.57 MΩ; SK2+/T: 305.46 ± 37.84 MΩ).

SK2 overexpression enhances SK channel mediated restriction of glutamatergic activity in CA1

We previously reported that blocking SK channels with apamin in CA1 neurons increased the amplitude of synaptically evoked glutamatergic-mediated EPSPs in an NMDAR-dependent manner (Ngo-Anh, Bloodgood et al. 2005). To determine whether the overexpression of SK2 channels would influence the magnitude of subthreshold synaptic events, 100-μs current pulses were applied to the stratum radiatum and whole-cell EPSPs were recorded from CA1 neurons in hippocampal slices from WT (Fig. 4.3a, b) and SK2+/T mice (Fig. 4.3c, d) before and after apamin application (100 nM). Summary plots reveal that in slices from WT mice, apamin increased the peak EPSP amplitude by an average of 59 ± 5% of the control (Fig. 4.3b), while in slices from SK2+/T mice apamin increased the peak EPSP amplitude by an average of 136 ± 8% of the control (Fig 4.3d and Table 4.1; $P < 0.02$).

These experiments were repeated using external solutions that contained lower concentrations of Mg^{2+} and higher concentrations of Ca^{2+}, conditions that augment NMDAR activation and further elevate Ca^{2+} influx through the NMDAR. Under these conditions blocking SK channels with apamin increased the EPSP amplitude by an average of 217 ± 12% of the control in slices from SK2+/T mice (Table 4.1), compared to an increase of 114 ± 11% of the control in WT slices ($P < 0.04$). Taken together these data indicate that the overexpressed SK2 channels enhance the SK channel-mediated restriction of NMDAR activation that limits synaptically evoked excitatory events.

SK2 overexpression attenuates hippocampal synaptic plasticity

Previously, we have shown that blocking SK channels with apamin altered the modification threshold with which synaptic plasticity was induced in mouse hippocampal slices (Stackman, Hammond et al. 2002). Therefore, we hypothesized that SK2 overexpression would affect the induction of synaptic plasticity. To test this, we stimulated CA3 Shaffer collateral synapses and recorded CA1 field potentials from hippocampal slices from SK2+/T mice and their WT littermates. Relative changes in synaptic strength were measured by the percent change in the maximum slope of fEPSPs. Induction of LTP with three 50 Hz tetani (100 pulses at 0.1 Hz) was significantly reduced in slices from SK2+/T mice (n = 14; 113.81 ± 3.67%), relative to WT slices 30 – 40 min after tetanization (n = 7; 133.06 ± 7.69%, $P = 0.018$; Fig. 4.4a). These data indicate that SK2 overexpression attenuates the induction of hippocampal LTP. Induction of LTP with three 100 Hz trains (each 100 pulses at 0.1 Hz) was equivalent in WT (n = 14; 137.42 ± 5.43%) and SK2+/T (n = 8; 130.94 ± 13.12%) slices 30-40 minutes after tetanization (Fig. 4.4b; $P > 0.05$). The magnitude of LTD induced by 1 Hz stimulation applied for 20 min was also equivalent in WT (n = 7; 80.73 ± 1.26%) and SK2+/T (n = 8; 76.58 ± 3.83%) slices 30-40 min after stimulation (Fig. 4.4c; $P > 0.05$). Taken together, these data indicate that SK2 overexpression impairs the induction of LTP in a frequency-dependent manner without affecting the induction of LTD.

To determine if these effects on synaptic plasticity could be explained by differences in CA1 basal synaptic transmission, we examined the input-output relationship of fiber volley amplitude to the field EPSP slope. Field EPSPs were recorded

from slices from WT (n = 5) and SK2+/T (n = 7) hippocampal slices over a range of stimulus intensities (2.5 - 10 μA) applied at 0.05 Hz. The maximum slope of the fEPSP was plotted versus the presynaptic fiber volley for each genotype (Fig. 4.5a). A three factor ANOVA revealed that presynaptic fiber volley amplitude was a significant predictor of fEPSP slope (fiber volley, $P < 0.001$), and no differences in this relationship were found between genotypes (genotype X fiber volley, $P > 0.05$). To determine if SK2 overexpression alters short-term plasticity mechanisms, we examined paired pulse facilitation (PPF) in WT (n = 5) and SK2+/T (n = 10) slices. Paired pulses were delivered at 0.1 Hz, separated by intervals of 12, 20, 50, 100, and 200 ms. Repeated measures ANOVA revealed no effect of genotype on the PPF ratio, calculated as the amplitude of second fEPSP divided by the amplitude of first fEPSP (Fig. 4.5b; interval X genotype, $P > 0.05$). This suggests that the mechanisms of paired-pulse facilitation, which are largely presynaptic (Zucker 1989), are not affected by SK2 overexpression. In addition, we examined possible effects of SK2 overexpression on the readily releasable pool of neurotransmitter vesicles by measuring the decline in fEPSPs over the 100 Hz tetanus. During this tetanus, the fEPSP will decline as the readily releasable pool of presynaptic vesicles is depleted. No differences were found between WT (n = 15) and SK2+/T (n = 8) slices in the decrease of the fEPSP slope of the 15th fEPSP relative to the 1st fEPSP (Fig. 4.5c; $P > 0.05$), further supporting the view that SK2 overexpression does not alter presynaptic neurotransmitter release mechanisms. Together, these data indicate that the effect of SK2 overexpression on synaptic plasticity is not due to altered presynaptic mechanisms.

SK2 overexpression severely impairs spatial learning and memory in the Morris water maze

Previously, we reported that systemic apamin enhanced encoding of spatial memory in the Morris water maze task; apamin-treated mice required fewer training trials to learn the location of the hidden platform than control-treated mice (Stackman, Hammond et al. 2002). Therefore, we hypothesized that SK2 overexpressing mice would require more training to learn the platform location in the water maze than WT mice. Prior to hidden platform training mice were first trained in a hippocampal-independent learning task (McDonald and White 1994); mice were trained for 2 days (6 trials/day) to swim to a visible platform that was cued with a protruding flag. Measures of the cumulative distance of the mouse to the target platform (CDT), reflecting platform search efficiency (Gallagher, Burwell et al. 1993) were recorded each trial. Although there was no significant genotypic difference in learning the visible platform task as assessed by CDT (genotype X trial block, $F_{2,29} = 2.01$; $P > 0.05$, Fig. 4.6a), a significant main effect of genotype was found for CDT measures ($F_{1,29} = 8.00$; $P = 0.008$). SK+/T mice also swam slower than WT mice in the visible platform task (Fig. 4.6b; genotype, ($F_{1,29} = 13.64$; $P = 0.001$) and when visible platform CDT data were reanalyzed with swim speed as a covariate, the main effect of genotype was not significant (ANCOVA: $F_{1,25} = 1.73$; $P > 0.05$). This result indicates that genotypic differences in CDT during visible platform training were due to differences in swim speed. Together the visible platform data indicate that SK2 overexpression does not obviously impair vision, swimming ability, or striatal-dependent learning (McDonald and White 1994).

In the hippocampal-dependent version of the Morris water maze task, mice use spatial cues to learn the fixed location of a hidden platform. Over successive training trials, mice encode a hippocampal-dependent spatial map that permits efficient and biased search behavior during subsequent probe tests. SK2+/T and WT mice were trained in the hidden platform task beginning 24 hr after completion of visible platform training. On each trial (4/day) mice were placed into the pool and allowed 60 sec to escape onto the hidden platform. During hidden platform training, SK2+/T mice exhibited significantly longer CDT measures (Fig. 4.6c; $F_{1,29} = 18.06$; $P < 0.001$) and longer escape latencies (not shown; $F_{1,29} = 19.25$; $P < 0.001$) compared to WT mice. Importantly, there were no genotypic differences in swimming speed in the hidden platform task (Fig. 4.6d; $F_{1,29} = 0.56$; $P > 0.05$). In addition, while WT mice attained asymptotic CDT scores by their 5th day of hidden platform training, the SK2+/T mice never reached a similar performance level even after 11 days of training (Fig. 4.6c). Although the performance of SK2+/T and WT mice improved at a similar rate over training (genotype X trial block, $F_{10,290} = 1.65$; $P > 0.05$), CDT scores differed considerably between genotypes at the conclusion of training. Together, these results demonstrate deficient spatial learning by SK2+/T mice and suggest that the SK2+/T mice were using a means of locating the hidden platform that was distinct from the spatial mapping strategy of their WT littermates.

SK2+/T mice exhibited longer CDT measures than WT mice ($t_{29} = 3.62$; $P = 0.001$) during the first day of hidden platform training. This initial difference in hidden platform task performance likely reflects an impairment of the SK2+/T mice in the efficiency of switching from the task requirements of the visible platform to those of the hidden platform task. In support of this view, a separate cohort of naïve SK2+/T (n = 12)

and WT littermate (n = 6) mice exhibited equivalent CDT scores on the first day of hidden platform training, when this training was not preceded by visible platform training (Fig. 4.7; WT: 11534.98 ± 2789.26 cm; SK2+/T: 12209.17 ± 1362.05 cm; $t_{16} = -0.247$; $P > 0.05$). As in the present study, this second cohort of SK2+/T mice exhibited impaired spatial learning and memory in the Morris water maze task.

The learning and memory impairments of SK2+/T mice were further revealed during the final probe test, administered 24 hours after the last training trial (Fig. 4.6e, f, g, h). WT mice exhibited a strong spatial bias in their search behavior during the final probe test, with 51.21 ± 4.43% of time spent in the quadrant of the pool where the platform was located during training (Fig. 4.6e), and a search ratio significantly above chance performance (Fig. 4.6f, $t_{16} = 5.80$; $P > 0.001$). Consistent with the quantitative analyses, representative traces of swim paths from the probe test indicate that WT mice used an allocentric spatial mapping strategy, conducting a spatially biased search in the region of the pool that previously contained the platform (Fig. 4.6g). However, even after this extensive training protocol, SK2+/T mice spent significantly less time in the quadrant of the pool where the platform was located during training (Fig. 4.6e, 31.15 ± 6.44%) than did WT mice ($t_{29} = 2.19$; $P = 0.01$). In addition, search ratio measures (Fig. 4.6f) indicate that SK2+/T mice exhibited significantly less accurate search behavior than WT mice ($t_{29} = 3.23$; $P = 0.003$) and failed to exhibit a significant spatial bias for the platform location ($t_{13} = 0.29$; $P > 0.05$). Representative swim paths of SK2+/T mice (Fig. 4.6h) further demonstrate the lack of an obvious spatial bias to their search behavior, and indicate that some mice exhibited an alternative egocentric search strategy (such as circular swimming a fixed distance from the wall of the pool). Such egocentric search

strategies are often used by water maze trained rodents with impaired hippocampal function (Morris, Garrud et al. 1982; Riedel, Micheau et al. 1999). These data suggest that SK2+/T mice failed to use a hippocampal-dependent spatial mapping strategy to find the hidden platform in the water maze. Together, these data demonstrate that SK2 overexpression severely impairs spatial learning and memory in the hippocampal-dependent version of the Morris water maze.

SK2 overexpression impairs contextual and cued fear conditioning

The contextual fear conditioning paradigm was used to further examine the effects of SK2 overexpression on hippocampal learning and memory. In contextual fear conditioning, the mouse forms an association between an aversive event and the distinctive context in which that event took place. Mice will exhibit conditioned fear responses when they are returned to the context, such as freezing (the absence of all movement except for respiration). This form of learning is dependent on the hippocampus (Kim and Fanselow 1992; Phillips and LeDoux 1992). WT (n = 29) and SK2+/T (n = 42) mice were first exposed to the conditioning chamber for a 5 min context pre-exposure session. During conditioning 24 hours later, mice were returned to the same chamber and received 3 pairings of a 30-s auditory tone and a 1-sec 0.5 mA footshock. Twenty-four hours after conditioning, mice were returned to the same chamber for a 5 min context test. Both genotypes exhibited conditioned fear to the context during the context test, expressed as an increase in % freezing relative to the pre-exposure session (Fig. 4.8; session, $F_{1,69} = 119.14$; $P < 0.001$). In addition, a significant genotype X session interaction was found ($F_{1,69} = 19.69$; $P < 0.001$), and post-hoc comparisons revealed that

SK2+/T mice exhibited significantly less % freezing to the context than WT mice (t_{69} = 4.49; $P < 0.001$) during the context test, indicating impaired contextual fear conditioning.

SK2 channels are also expressed throughout the amygdala and may modulate amygdala-dependent learning and memory. Lesions of the amygdala disrupt fear conditioning to auditory stimuli (Phillips and LeDoux 1992). Therefore, we also examined retention of amygdala-dependent cued fear conditioning in SK2+/T and WT littermate mice. One hour after the context test, mice were placed into a novel environment for the tone test, in which they were presented with the tone after a 1 min delay. Both genotypes exhibited conditioned fear to the tone, expressed as increased % freezing in min 2 (after the tone presentation) relative to min 1 (prior to the tone presentation) (Fig. 4.8; min, $F_{1,69} = 28.66$; $P < 0.001$). A significant genotype X min interaction was also found ($F_{1,69} = 8.69$; $P = 0.004$), and post-hoc comparisons revealed that SK2+/T mice exhibited less freezing in response to the tone (min 2) than WT mice (Fig. 4.8; $t_{69} = 2.90$; $P = 0.005$), suggesting that SK2 overexpression impaired cued fear conditioning. Importantly, no differences in % freezing were found between genotypes during the first minute of the tone test (prior to tone presentation), indicating that SK2 overexpression did not affect baseline freezing behavior (Fig. 4.8; $t_{69} = 0.20$; $P > 0.05$).

To ensure that SK2+/T mice were equally sensitive to the acoustic and footshock stimuli used in this delay fear conditioning procedure, separate cohorts of mice (WT: n = 6-7, SK2+/T: n = 6) were tested for acoustic startle response and shock sensitivity. Repeated measures ANOVA revealed no effect of genotype on the percent change in the peak response with a 60 dB (WT 123.85 ± 2.13 %, SK2+/T 120.66 ± 2.38 %), 80 dB (WT 142.12 ± 3.34 %, SK2+/T 144.16 ± 3.17 %), or 100 dB (WT 142.88 ± 2.76 %,

SK2+/T 146.99 ± 3.57 %) tone, indicating that tone salience was equivalent for SK2+/T and WT mice ($F_{1, 198} = 0.09$; $P > 0.05$). The shock threshold (in mA) required to elicit a response, determined for each mouse during shock sensitivity testing, were not different between genotypes (WT 0.32 ± 0.03 mA, SK2+/T 0.32 ± 0.04 mA, $t_{10} = 0.37$; $P > 0.05$). Taken together, these data show that SK2 overexpressing mice have deficits in memory for hippocampal- and amygdala-dependent conditioned fear responses. Importantly, these deficits are not likely due to sensory or motor differences between the genotypes.

Discussion

Blocking SK channels with apamin enhances hippocampal synaptic plasticity (Behnisch and Reymann 1998; Stackman, Hammond et al. 2002; Kramar, Lin et al. 2004) and memory encoding (Stackman, Hammond et al. 2002). The present study demonstrates that SK2 channels selectively regulate the hippocampal physiology and synaptic plasticity that may underlie spatial and contextual memory formation. SK2 overexpression increased the apamin-sensitive current in hippocampal CA1 neurons, decreased synaptically evoked glutamatergic EPSPs, attenuated 50 Hz LTP in hippocampal slices, and drastically impaired learning and memory in two hippocampal-dependent tasks. SK2 overexpression did not alter basal synaptic transmission or presynaptic release mechanisms (Fig. 4.4). These effects are not likely due to differences in the SK2 expression pattern since the endogenous promoter drives expression in SK2+/T mice, nor are these effects due to compensatory changes in SK1 or SK3 channel expression (Fig. 4.1b).

In agreement with the observed frequency-dependent impairment of LTP induction, we found that SK2 overexpression disrupted hippocampal-dependent learning and memory in both the Morris water maze (Fig. 4.6) and a contextual fear-conditioning paradigm (Fig. 4.8). In the Morris water maze, SK2+/T mice exhibited profound learning impairments, and were unable to learn and retain the platform location even after 11 days of training. Cumulative distance to platform measures of the SK2+/T mice did improve over the course of hidden platform training, yet not to the degree of the WT mice. Examination of behavior of SK2+/T mice during the final probe test revealed a marked lack of the characteristic biased search in the location of the pool that previously

contained the platform. These data are consistent with the view that SK2 channel overexpression may have prevented the hippocampal-based encoding and retention of a spatial representation or map of the platform's position with respect to extra-maze cues. It is unlikely that the observed effects were due to impaired sensorimotor function, since SK2+/T mice were able to learn to locate a visible platform in the striatal-dependent water maze task. SK2 overexpression disrupted both hippocampal-dependent contextual fear conditioning and hippocampal-independent cued fear conditioning. These impairments are not likely due to sensorimotor differences since SK2+/T mice exhibited baseline freezing behavior, acoustic startle responses, and shock sensitivities equivalent to that of WT mice. The impairment of SK2+/T mice in cued fear conditioning, an amygdala-dependent task (Davis 1994), is consistent with the fact that SK2 channels are expressed throughout the amygdala (Sailer, Hu et al. 2002; Sailer, Kaufmann et al. 2004). Furthermore, cued fear conditioning has been associated with LTP of the lateral amygdala (Rogan and LeDoux 1995; Rogan, Staubli et al. 1997) and SK channels in the lateral amygdala regulate the activity of NMDA receptors (Faber, Delaney et al. 2005)-providing a similar mechanism for SK channel-mediated regulation of amygdala-dependent synaptic plasticity and learning. A number of previous studies have revealed that treatment of rats and mice with the SK channel antagonist apamin can facilitate the acquisition of a number of learning tasks (Tzounopoulos and Stackman 2003). We previously found that apamin facilitated the encoding of hippocampal memory in both spatial and object memory tasks (Stackman, Hammond et al. 2002). The present findings suggest that overexpression of SK2 channels may delay acquisition of hippocampal-

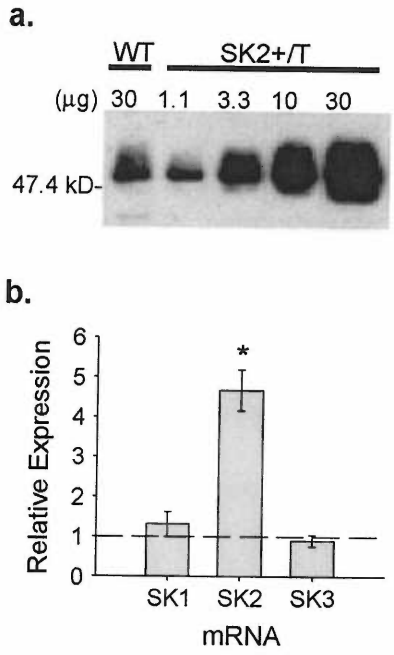
dependent memory and that SK2 channels likely contribute to the cognitive effects of apamin.

There are at least two mechanisms by which the overexpression of SK2 channels might affect hippocampal function. Whole-cell recordings revealed that the apamin-sensitive current in CA1 neurons, which contributes to the afterhyperpolarization, was significantly greater in SK2 overexpressing mice than in WT mice. The increase in the apamin-sensitive current in CA1 is likely to affect neuronal excitability at the level of the soma, an influence that has been shown to influence learning and memory in several behavioral paradigms (Disterhoft, Golden et al. 1988; McEchron, Weible et al. 2001; Oh, Kuo et al. 2003). A second, and more direct mechanism by which SK2 channels may affect the induction of synaptic plasticity is via a Ca^{2+}-mediated feedback loop between NMDA receptors and SK channels in the dendritic spines of CA1 neurons, residing within 25-50 nm (Ngo-Anh, Bloodgood et al. 2005). The 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). The synaptically evoked Ca^{2+} influx activates SK channels located within 25-50 nm of NMDA receptors in the spine (Ngo-Anh, Bloodgood et al. 2005). The resulting SK channel activation partially shunts the AMPA receptor-mediated depolarization, favoring the voltage-dependent Mg^{2+} block of NMDA receptors and thereby attenuating Ca^{2+} influx. In this model, SK2 overexpression might be expected to increase the SK component of the EPSP, and indeed, we found that the SK channel-mediated restriction of glutamatergic EPSPs was significantly enhanced compared to wild type controls (Fig. 4.3). Therefore, the overexpressed SK channels are distributed to the dendritic spine compartments where

they couple to NMDAR Ca^{2+} influx. Moreover, these results are consistent with the proposed mechanism whereby SK channels regulate the induction of synaptic plasticity through the modulation of NMDAR activation and suggest that these cellular mechanisms underlie the observed alterations in hippocampal-dependent memory.

These results extend previous findings that SK channels regulate hippocampal synaptic plasticity, learning, and memory, and provide evidence that SK2 channels mediate these processes. However, we cannot conclude from these data that homomeric SK2 channels are the only SK channels that contribute to the regulation of hippocampal function. SK3 and SK1 channels are also expressed in the rodent hippocampus, albeit at lower levels than SK2 channels (Stocker and Pedarzani 2000; Tacconi, Carletti et al. 2001; Sailer, Hu et al. 2002). There is evidence that SK3 channel expression levels influence memory (Blank, Nijholt et al. 2003). However, transgenic mice with conditional overexpression or knockdown of SK3 (Bond, Herson et al. 2004) do not exhibit altered hippocampal-dependent spatial memory in the Morris water maze (unpublished results). In addition, SK3 channels have been shown to be localized to presynaptic terminals in cultured mouse hippocampal neurons (Obermair, Kaufmann et al. 2003), suggesting that any SK3 contribution to hippocampal function would occur through presynaptic mechanisms. Further, the apamin-sensitive current in CA1 neurons is abolished in SK2-null mice, yet not significantly affected in mice lacking SK1 or SK3 channels (Bond, Herson et al. 2004). In conclusion, the results from the present study indicate that SK2 channel subunits specifically play an important role in the modulation of hippocampal synaptic plasticity, learning and memory.

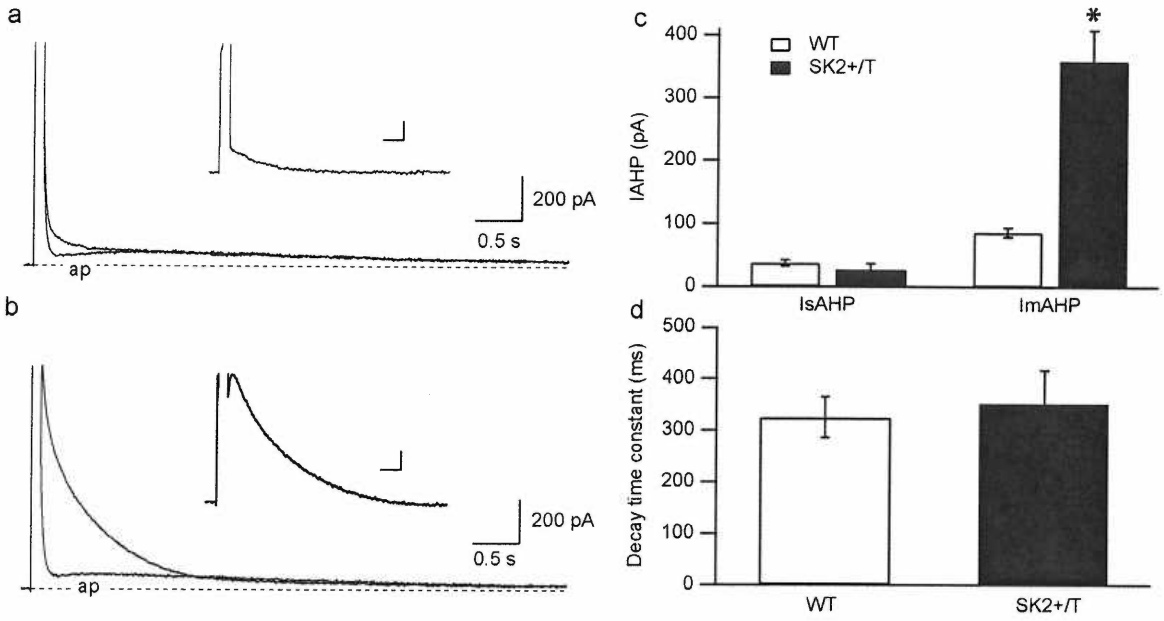
Figure 4.1.



SK2+/T mice specifically overexpress SK2 channels.

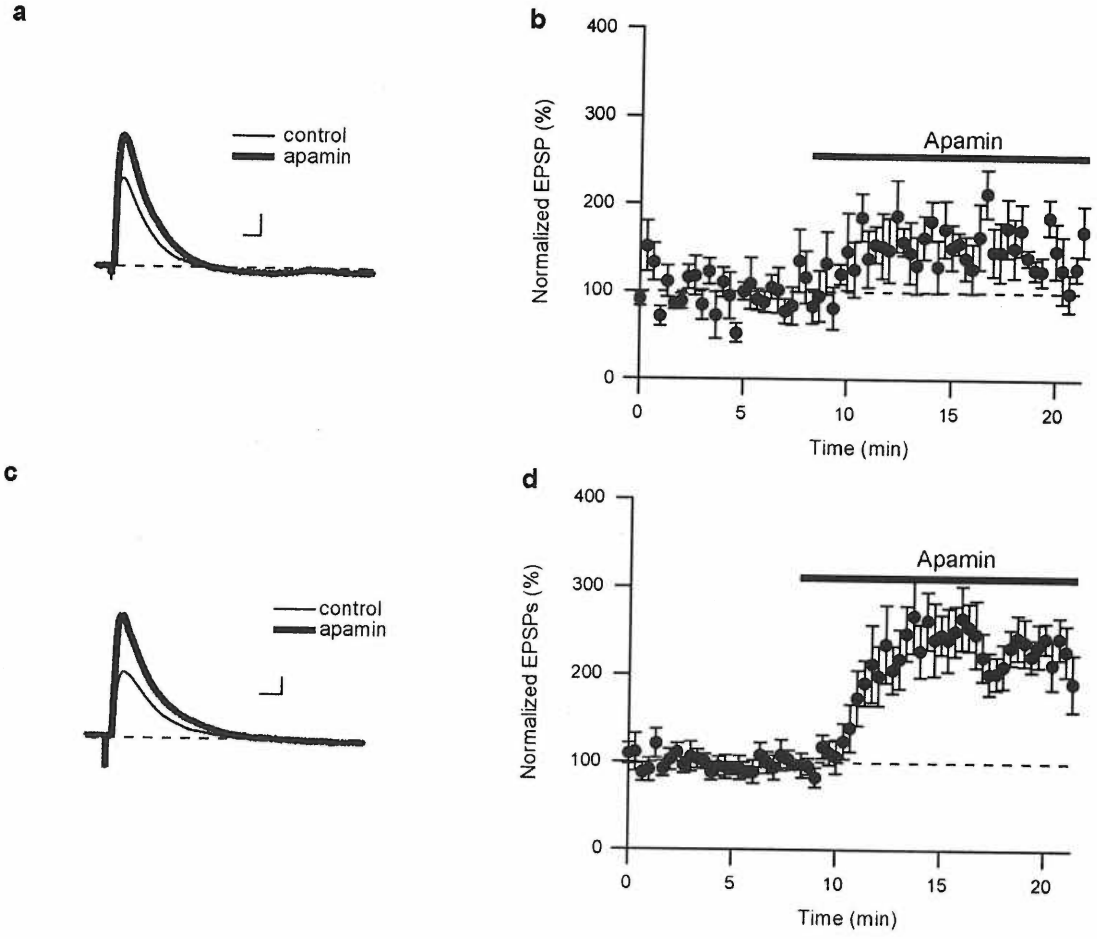
(a) Western blot reveals approximately a 10-fold overexpression of SK2 channels in SK2+/T mice. Membranes were prepared from whole brain homogenate from WT or SK2+/T mice. Increasing amounts of brain membrane proteins from SK2+/T mice (1.1 μg, 3.3 μg, 10 μg, 30 μg) were loaded onto the gel for semi-quantitative analysis. **(b)** Real-time PCR reveals that SK2 transcripts are overexpressed by approximately 4.5-fold, while SK1 and SK3 transcripts are expressed at WT levels in SK2+/T mice. Error bars, s.e.m. * $P < 0.05$.

Figure 4.2.



SK2 overexpression increases the medium component of the apamin-sensitive current. Representative whole cell recordings of CA1 neurons from WT (**a**) and SK2+/T (**b**) mice before and after apamin (ap) are shown. Cells were voltage clamped at –55 mV and tail currents were elicited following a 100 ms depolarizing step to +20 mV. (calibration brackets: 200 pA, 0.5 ms). Insets reflect the subtracted apamin-sensitive current (calibration brackets: 100 pA, 200 ms). (**c**) Summary plot reveals that SK2 overexpression increases the amplitude of the apamin-sensitive current measured at 100 ms (ImAHP) by approximately 4-fold, but does not affect the apamin-insensitive current measured at 1 s (IsAHP). (**d**) SK2 overexpression does not alter the decay kinetics of the ImAHP. Error bars, s.e.m. * $P < 0.05$.

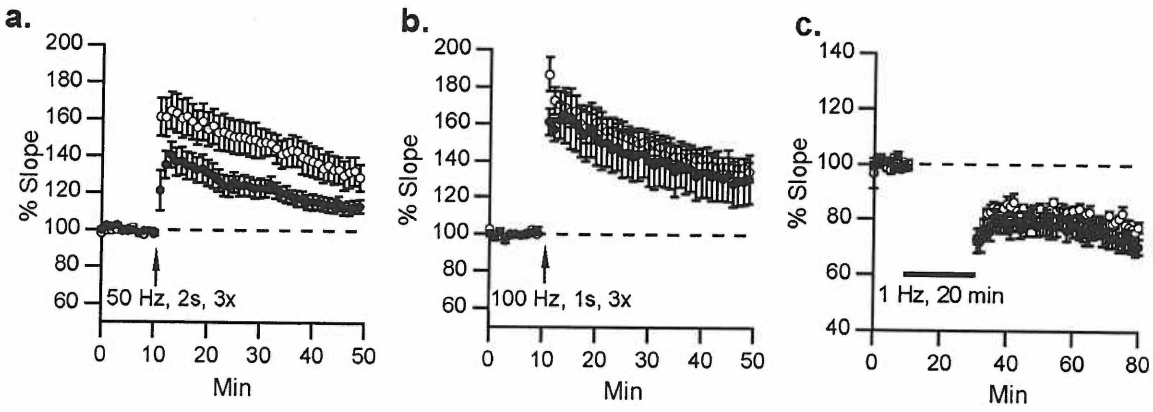
Figure 4.3.



SK2 overexpression enhances the SK channel-mediated attenuation of the synaptically evoked glutamatergic EPSPs in CA1.

An average EPSP waveform was derived from 20 EPSPs synaptically evoked in control condition in WT (**a**) and SK2+/T mice (**c**) before and after application of apamin. (**b**) Summary plot of the EPSP amplitude of WT mice under control condition relative to the baseline period during wash-in of apamin. (n = 5 cells). (**d**) Summary plot of the EPSP amplitude of SK2+/T mice under control condition relative to the baseline period during wash-in of apamin. (n = 7 cells). (Calibration brackets for **a** and **c**: 1 mV, 25 ms). The times of drug application are indicated by the horizontal bars. All error bars, s.e.m.

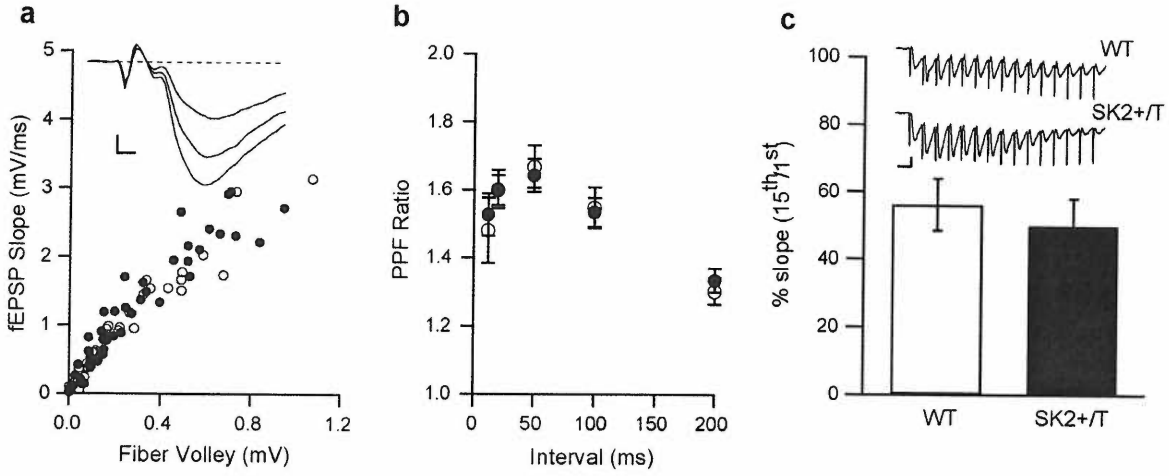
Figure 4.4.



SK2 overexpression impairs the induction of synaptic plasticity in a frequency-dependent manner.

Field potentials were recorded from the CA1 region of WT (○) and SK2+/T (●) hippocampal slices. (**a**) LTP was induced with 3 tetani consisting of 100 pulses delivered at 50 Hz (0.1 Hz). Slices from SK2+/T mice exhibited significantly less LTP 30 - 40 min after 50 Hz stimulation than slices from WT mice ($P = 0.018$). (**b**) SK2 overexpression did not disrupt 100 Hz LTP induced with 3 tetani consisting of 100 pulses delivered at 100 Hz (0.1 Hz). (**c**) SK2 overexpression did not disrupt LTD induced with a low frequency stimulation of 1 Hz for 20 min. All error bars, s.e.m.

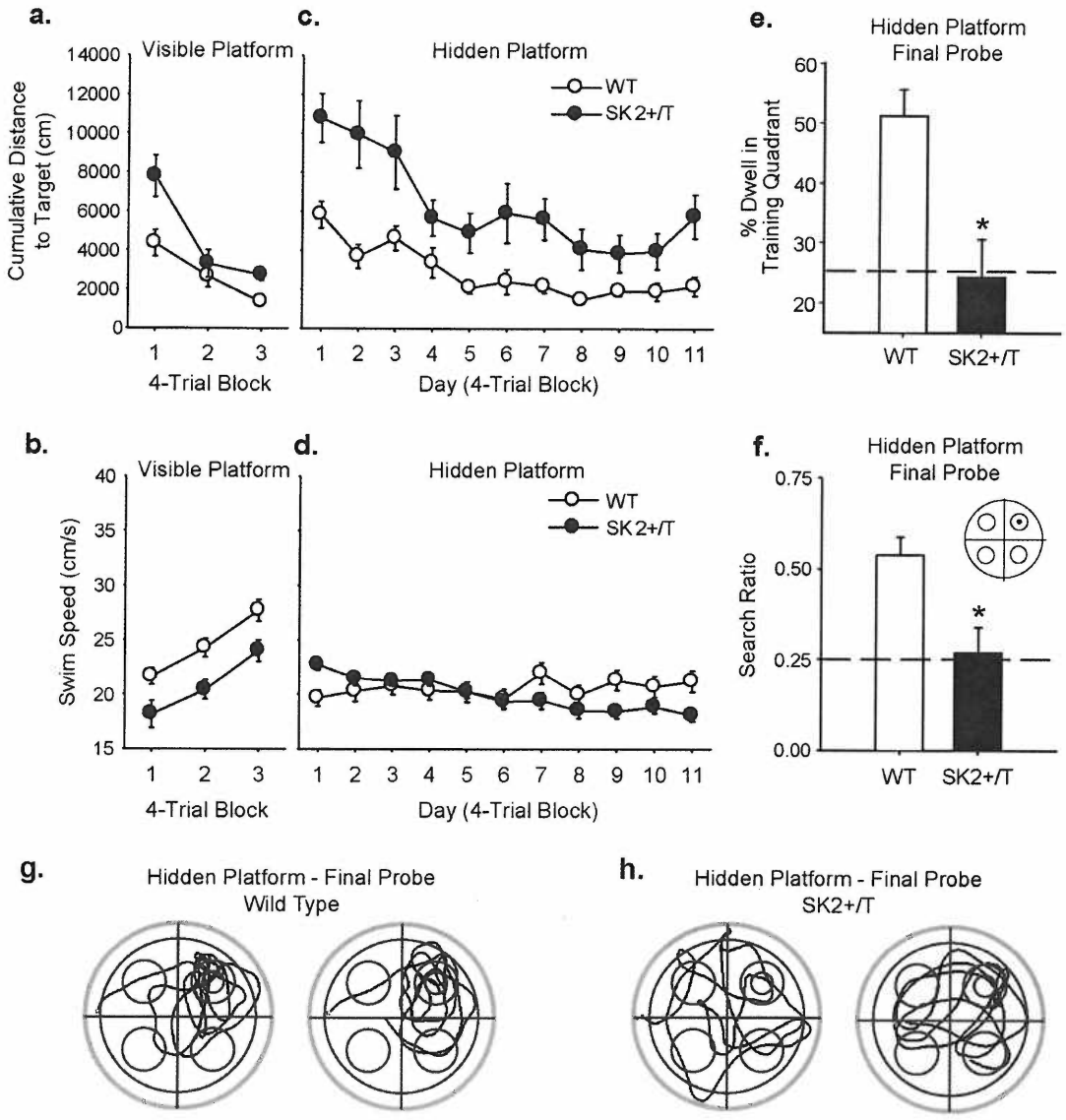
Figure 4.5.



SK2 overexpression does not alter basal synaptic transmission or presynaptic release mechanisms.

(**a**) Field EPSP slopes were plotted against the corresponding fiber volley amplitude. The inset depicts representative traces from an SK2+/T slice (calibration bracket: 0.25 mV, 2 ms). For each genotype, fiber volley amplitude was a significant predictor for fEPSP slope ($P< 0.001$), and there was no genotype effect on this relationship ($P > 0.05$), indicating that SK2 overexpression does not alter basal synaptic transmission properties. WT (○) and SK2+/T (●). (**b**) SK2 overexpression did not alter the paired pulse facilitation (PPF) ratio ($P > 0.05$). (c) Decline in fEPSPs during 100 Hz stimulation was not altered by SK2 overexpression ($P > 0.05$). WT (○) and SK2+/T (●). Inset depicts representative traces (calibration bracket: 0.25 mV, 5 ms). All error bars, s.e.m.

Figure 4.6.

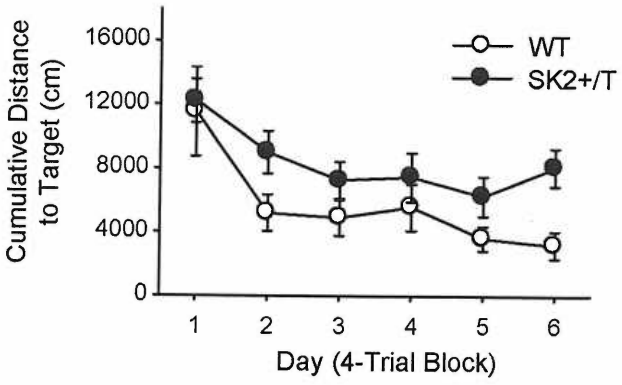


SK2 overexpression impairs hippocampal-dependent learning and memory in the Morris water maze.

Learning in the hippocampal-independent visible platform water maze task is not different between WT (○) and SK2+/T (●) mice as assessed by CDT with swim speed as a covariate (**a**), ANCOVA ($P > 0.05$), although SK2+/T mice swam slower than WT

mice (**b**). Learning in the hippocampal-dependent hidden platform water maze is significantly impaired in SK2+/T mice relative to WT mice (**c**, $P < 0.001$), and there were no genotypic differences in swim speed in this version of the task (**d**, $P > 0.05$). (**e**) During the final probe test 24 hr after the last hidden platform training trial, SK2+/T mice spent significantly less time in the quadrant of pool relative to the WT mice ($P < 0.04$). (**f**) Search ratios computed from final probe test data indicate that SK2+/T mice failed to show a spatial bias for the platform location; search ratios were equivalent to chance performance ($P > 0.05$) and were significantly lower than WT ratios ($P = 0.003$), indicating that SK2 overexpression severely restricts learning and remembering the location of the hidden platform. Search ratios were defined as the frequency of crossings through a circular zone around the platform location (**see inset, f**) divided by the frequency of crossings into all 4 circular zones. Representative tracings of swim paths of two WT mice (**g**) and two SK2+/T mice (**h**) during the final probe test. All error bars, s.e.m. * $P < 0.05$.

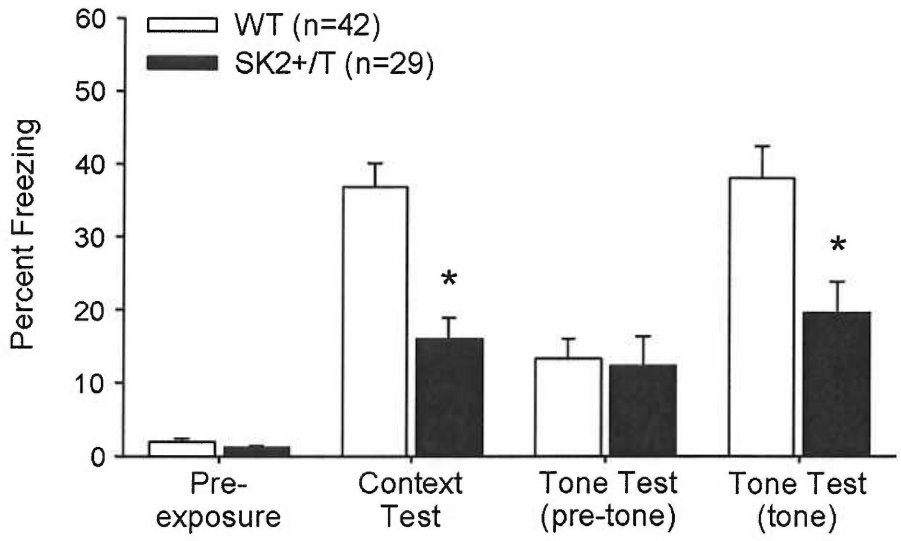
Figure 4.7.



SK2 overexpression impairs learning and memory in the Morris water maze.

In this experiment, naïve male SK2+/T (n=12) and WT littermate mice (n=6) were trained in the hidden platform water maze task for 6 days (4 trials/day). Naïve SK2+/T and WT mice exhibited similar CDT measures on the first day of training, suggesting that the differences between SK2+/T and WT mice in CDT scores (in Fig. 6) on the first day of hidden platform training may be due to prior experience in the visible platform task.

Figure 4.8.



SK2 overexpression impairs contextual and cued fear conditioning.

There was no genotypic difference in freezing behavior between WT and SK2+/T mice during the 5 min context pre-exposure session. During the context test 24 hr after conditioning, both genotypes exhibited conditioned fear demonstrated by increased freezing relative to the pre-exposure session ($P < 0.001$), although % freezing was significantly reduced in SK2+/T mice compared to WT controls ($P < 0.001$), indicating impaired contextual conditioning in SK2+/T mice. During the tone test, before the tone was presented (min 1), there were no differences in % freezing between genotypes ($P > 0.05$), indicating SK2 overexpression did not alter baseline freezing behavior. However, during the tone test SK2+/T mice exhibited less freezing in response to the tone compared to WT mice (min 2; $P = 0.004$), indicating that SK2 overexpression also impaired cued fear conditioning. All error bars, s.e.m. * $P < 0.05$.

Table 4.1

Genotype	Tx (*n*)	EPSP (mV)		Slope (mV/ms)		Half-width (ms)	Rise time (ms)
WT	Control	2.8 ± 0.2		0.67 ± 0.04		45 ± 1.4	3.9 ± 0.2
	Apamin (*5*)	4.6 ± 0.4	159 ± 5%	0.98 ± 0.1	139 ± 7%	45 ± 2.04	4.2 ± 0.1
SK2+/T	Control	3.3 ± 0.08		0.69 ± 0.04		51 ± 1.2	4.2 ± 0.1
	Apamin (*7*)	7.6 ± 0.2	236 ± 8%[a]	1.43 ± 0.05	228 ± 13%[a]	53 ± 1.8	4.9 ± 0.2
WT	Control (Low Mg^{2+})	4.2 ± 0.2		0.81 ± 0.04		43 ± 2.7	5.0 ± 0.2
	Apamin (*6*)	8.5 ± 0.4	214 ± 12%	1.72 ± 0.08	216 ± 5%	42 ± 2.8	4.3 ± 0.2
SK2+/T	Control (Low Mg^{2+})	3.0 ± 0.1		0.63 ± 0.05		49 ± 2.0	4.7 ± 0.2
	Apamin (*6*)	9.2 ± 0.5	318 ± 12%[a]	1.77 ± 0.13	327 ± 16%[a]	46 ± 1.9	4.5 ± 0.4

Mean ± S.E.M. properties of synaptically evoked EPSP responses in hippocampal slices from WT and SK2+/T mice. Slope was defined as the maximum rate of rise of the EPSP. Half-width (ms) refers to the width of the EPSP measured at 50% of the maximum amplitude. Rise time refers to the time required for the EPSP to rise from 20% to 80% of the maximum amplitude. The percentage of control values for each condition is presented in parentheses.

[a] $P < 0.05$ compared to the respective value from WT slices.

V. DISCUSSION AND CONCLUSIONS

In humans and rodents, the hippocampus is an essential structure for learning and memory processes. The work presented here demonstrates that SK channels are an integral part of the neurobiological mechanisms regulating hippocampal functions, including synaptic plasticity, learning, and memory. A variety of techniques were utilized throughout this study to examine the role of SK channels in hippocampal processes. Intra-hippocampal lidocaine administration was used in section II to determine the contribution of hippocampal activity to object recognition memory. In section III, systemic and intra-hippocampal apamin administrations were used to determine the role of SK channels in object memory processes. Finally, in section IV, electrophysiological recordings were used to assess the effects of SK2 overexpression on hippocampal synaptic transmission and synaptic plasticity, and behavioral testing (including the Morris water maze and contextual fear conditioning) was utilized to examine the effects of SK2 overexpression on hippocampal learning and memory processes.

Hippocampal involvement in object recognition memory

To assess the involvement of the hippocampus in object recognition memory, intra-hippocampal lidocaine administration was used to reversibly inactivate the CA1 region of the dorsal hippocampus (section II of this thesis). Hippocampal inactivation prior to training impaired object recognition memory after a long (24 hour) retention interval, but not after a short (5 min) retention interval. These data are consistent with the current literature, which suggests that there is a delay-dependent requirement of

hippocampal activity for novel object discrimination in mice. However, this does not conclude that the hippocampus is only involved in object memory processing during long retention intervals.

A more reasonable interpretation of the data is that hippocampal activity is preferentially involved in recollection-like over recognition-like behaviors (Fortin, Wright et al. 2004). In this case, hippocampal activity would be specifically involved in the encoding of object information necessary for later recollection (in humans, this refers to conscious recall of the declarative memories of the encountered object) but not processes of recognition (in humans, this would be recognizing an object as familiar without a conscious recollection of the object encounter). In section II of this thesis, hippocampal inactivation did not alter novel object preference when a short (5 min) retention interval was imposed. It is possible that object recognition-like processes are mediated by extra-hippocampal structures, and these processes guide novel object preference after short retention intervals. In fact, previous studies have shown that extrahippocampal structures of the medial temporal lobe, in particular the inferior temporal cortex (TE) and perirhinal cortex, are critical for object recognition processes in rodents (Winters and Bussey 2005) and primates (Meunier, Bachevalier et al. 1993; Murray and Richmond 2001). Importantly, the TE and perirhinal cortices mediate both processes of object perception and object memory. Neuronal activation studies in monkeys have shown that the firing properties of neurons in these regions are directly related to the familiarity or novelty of visual stimuli (Xiang and Brown 1999). Therefore, the firing properties of these cortical areas could be mediating short-delay novel object recognition-like processes through the encoding of object familiarity and novelty.

On the other hand, object recollection-like processes mediated by the hippocampus may guide novel object preference after long retention intervals. Consistent with this, results from section II of this thesis demonstrate that hippocampal inactivation disrupts novel object preference when a long (24 hour) retention interval is imposed. Therefore, the observed delay-dependent involvement of the hippocampus in object recognition memory is simply a consequence of two separate processes (recognition and recollection) mediating the same behavior (novel object preference). It is important to note here that it is unclear whether these two processes occur in parallel. While recognition-like processes may guide novel object preference after short retention intervals, recollection-type processes may guide novel object preference after long retention intervals. This seems unlikely though, considering that in humans, recollection occurs regardless of retention interval.

It is more likely that hippocampal-mediated recollection-like processes are utilized regardless of retention interval. In this case, hippocampal inactivation does not impair short-delay novel object preference because recognition-like processes (guided by cortical structures) are intact and mask the impairment. However, this interpretation suggests that (cortically mediated) recognition-like processes are more temporary than (hippocampally mediated) recollection-like processes; otherwise the object recognition impairment would also be masked with long retention intervals imposed. While neurons in the TE and perirhinal cortices have been shown to code for object familiarity and novelty, these neuronal properties were reported to last over ~24 hours (Xiang and Brown 1999). Regardless, the ability of these cortical regions to mediate novel object preference may still be delay-dependent. Together, results from section II of this thesis demonstrate

that with long retention intervals imposed, the object recognition task is useful for the examination of hippocampal-dependent learning and memory processes.

SK channel regulation of object memory encoding

As described above, section II of this thesis demonstrates that the spontaneous object recognition task is a valuable paradigm for examining rodent models of declarative memory. Therefore, this task was used to assess hippocampal-dependent learning and memory processes in section III of this thesis. Results from section III indicate that SK channels are involved specifically in object memory encoding processes, but not object memory retention processes. These data nicely complement previous studies examining the role of SK channels in hippocampal-dependent learning and memory using the Morris water maze (Stackman, Hammond et al. 2002). In this study, apamin-treated mice required less training in the Morris water maze than control mice to learn the location of a hidden platform. Likewise, results from section III of this thesis indicate that apamin-treated mice require less training to exhibit novel object preference in the object recognition task.

Since blockade of SK channels with apamin enhances memory encoding, these studies suggest that SK channel activation suppresses hippocampal-dependent memory encoding. Specifically, it is hypothesized that SK channels regulate hippocampal functions through their attenuation of NMDAR activation (see below discussion: "working model"). This model would predict that in the absence of SK channel modulators (i.e.: under basal conditions) SK channels in the hippocampus are actively suppressing memory encoding processes. While it may seem counter-intuitive that the

brain contains a system to limit hippocampal functions- specifically learning and memory processes critical for survival- it is likely that the role of SK channels in suppression of learning and memory processes is itself vital for the functioning of the system as a whole. Throughout biology there are many examples of molecular mechanisms responsible for maintaining the homeostasis of a system, such as in the regulation of temperature, osmolarity, or pH, to name a few. Likewise, mechanisms regulating hippocampal functions also require an antagonistic component to balance the system. In this manner, SK channels serve to modulate learning and memory processes, possibly through maintaining a set point allowing for the continuous encoding of new information. This is most likely achieved via the direct modulation of hippocampal synaptic plasticity by SK channels. Details of these mechanisms are discussed below (see "SK2 channel regulation of hippocampal function").

SK2 channel regulation of hippocampal function

Results from section IV of this thesis demonstrate that SK2 channels regulate hippocampal synaptic plasticity, learning, and memory. In fact, previous studies with apamin, the selective SK channel blocker, suggest that SK channels regulate the induction of hippocampal synaptic plasticity, learning and memory (Stackman, Hammond et al. 2002). In addition, in section III of this thesis, studies using apamin also support the hypothesis that SK channels regulate non-spatial hippocampal-dependent learning and memory processes. Specifically, in section IV, SK2 overexpression reduced LTP elicited with 50 Hz high frequency stimulation. However, SK2 overexpression did not disrupt LTP elicited with 100 Hz high frequency stimulation, nor did it disrupt LTD

elicited with 1 Hz low frequency stimulation. Therefore, the specific effect of SK2 channel overexpression on plastic events was observed after a relatively intermediate stimulus frequency (50 Hz). This suggests that SK2 channel regulation of synaptic plasticity may serve as a mechanism to maintain the direction of synaptic plasticity (LTP or LTD).

The threshold of crossover between LTD and LTP is known as the *modification threshold* (θ_m), and was first described by Bienenstock, Cooper, and Munro (1982). In this model, the θ_m is described as sliding along the bidirectional range of synaptic plasticity. Alterations in θ_m occur in an activity-dependent manner, and serve to regulate the direction of synaptic plasticity (e.g.: LTP or LTD). The sliding function of θ_m may therefore be an important mechanism for ensuring that, regardless of prior experience, synapses are capable of maintaining a dynamic range of responses. This regulation of synaptic plasticity has been coined "metaplasticity" (Abraham and Bear 1996). Without metaplasticity, a synapse receiving repeated LTP-inducing stimuli would continuously potentiate to saturation, eliminating its ability to respond to subsequent stimuli (this would also be true for repeated LTD-inducing stimuli, with subsequent saturation of LTD). Because SK2 channels alter synaptic plasticity close to θ_m, it is possible that their primary function is to mediate metaplasticity in the hippocampus, so that with SK2 channel modulation of θ_m, the bidirectional response range of the synapse can be maintained.

The behavioral consequences of SK2 channel modulation of synaptic plasticity in the hippocampus have also been examined in this thesis. Results from section IV indicate that SK2 channel overexpression severely impairs hippocampal-dependent learning and

memory. Specifically, SK2 channel overexpression impaired learning the location of the hidden platform in the Morris water maze, a hippocampal-dependent task. In addition, SK2 overexpression impaired contextual fear conditioning, which also requires an intact hippocampus. Together, these data indicate that SK2 channels regulate hippocampal-dependent learning and memory processes. In addition, these data nicely compliment previous findings; while studies with apamin have shown SK channel down regulation (via blockade by apamin) enhances learning and memory in hippocampal-dependent tasks (Stackman, Hammond et al. 2002), the present studies demonstrate that SK channel up regulation (via SK2 overexpression) impairs learning and memory in hippocampal-dependent tasks (Hammond, Bond et al. 2005).

Results from section IV of this thesis provide new evidence that primarily SK2 channels mediate the regulation of hippocampal synaptic plasticity, learning, and memory. Other studies have also implicated SK2 channels as the SK channel subtype responsible for regulating neuronal processes. SK2 channels alone are necessary for the apamin-sensitive current in CA1 neurons (Bond, Herson et al. 2004). In addition, studies with Lei-Dab7 (Shakkottai, Regaya et al. 2001), an SK2 channel specific antagonist, indicate that SK2 channels are involved in hippocampal synaptic plasticity, with application of Lei-Dab7 enhancing hippocampal LTP elicited with theta bust stimulation (Kramar, Lin et al. 2004). However, it remains possible that other SK channel subtypes also contribute to these processes. For example, SK3 channels may be capable of regulating memory processes (Blank, Nijholt et al. 2003). In this study, SK3 overexpression impaired hippocampal LTP and hippocampal-dependent learning and memory in young mice. However, in preliminary studies in our lab using SK3

conditionally-expressing transgenic mice (Bond, Sprengel et al. 2000), we have not been able to detect an effect of SK3 knockdown or SK3 overexpression on hippocampal-dependent memory using the Morris water maze (Hammond, Tull et al. 2002). Furthermore, SK3 channels are reported to be localized to the presynaptic terminals in mouse hippocampal neurons (Obermair, Kaufmann et al. 2003), suggesting that any SK3 channel regulation of hippocampal function would occur through a presynaptic mechanism. In addition, results from section IV of this thesis clearly support the hypothesis that SK2 channels regulate hippocampal function through postsynaptic mechanisms, since no effect of SK2 overexpression was found on the paired pulse facilitation ratio. Specifically, a working model detailing how SK2 channels modulate hippocampal function has evolved throughout the studies of this thesis. This working model is detailed below.

Working model

In the CA1 region of the hippocampus, both LTP and LTD require NMDAR activation. Therefore, SK channel modulation of NMDAR activity may be an important mechanism in the regulation of hippocampal synaptic plasticity and behavior. In this model, SK2 channels influence hippocampal processes through their direct modulation of postsynaptic NMDA receptors. As illustrated in Fig 5.1, this model requires that in CA1 neurons, SK2 channels are located in close proximity to NMDA receptors within the postsynaptic density. During synaptic stimulation, inward Na^+ current through AMPA receptors depolarizes the membrane and removes the Mg^{2+} block of NMDA receptors. Subsequently, NMDA receptors are activated by glutamate and conduct Ca^{2+} into the

spine. This increase in spine Ca^{2+} in turn activates SK2 channels, which conduct an outward K^+ current, repolarizing the membrane. With membrane repolarization, the Mg^{2+} block of NMDARs is reinstated, and therefore further NMDAR activation is attenuated. Therefore, this model predicts that membrane repolarization (resulting from SK2 channel activation) is the physiological switch by which SK2 channels modulate the activation of synaptic NMDARs.

With respect to hippocampal metaplasticity, this model is elegant because it provides a mechanism by which SK2 channels may act to modulate θ_m. In the CA1 region of the hippocampus, both LTP and LTD are NMDAR-dependent. Therefore, through regulation of NMDAR activation, SK2 channels may moduate the direction of synaptic plasticity. In fact, computational modeling has suggested that the Ca^{2+} buffering properties of the dendritic spine could account for the sliding of θ_m (Gold and Bear 1994). It is easy to imagine that with repeated LTP- or LTD-inducing stimuli, Ca^{2+} through NMDARs is activating a larger proportion of SK2 channels, which limits further NMDAR activation. Because the properties of the Ca^{2+} transient are responsible for the direction of plasticity (with brief, large increases in spine Ca^{2+} resulting in LTP, and prolonged, modest increases in spine Ca^{2+} resulting in LTD), it seems plausible that once activated, SK channels would limit the further development of plasticity in the direction consistent with the properties of the Ca^{2+} transient. For example, LTP-inducing stimuli resulting in large, brief increases in spine Ca^{2+} activate SK2 channels, which limit NMDAR activation, and suppress the further development of LTP (consequently setting θ_m closer to an LTD range). In this manner, SK2 channels may play a key role in hippocampal metaplasticity.

Computational modeling has also shown that shifts in NMDAR conductance due to differential subunit composition may also effect θ_m (Castellani, Quinlan et al. 2001). In hippocampal neurons, individual spines vary in their NMDAR subunit compostition and Ca^{2+} current properties (Sobczyk, Scheuss et al. 2005). In addition, NMDARs with distinct subunits differentially contribute to the induction mechanisms of LTP and LTD. Antagonists that specifically target NMDARs containing NR2A/B subunits preferentially block LTP but not LTD (Hrabetova, Serrano et al. 2000). Therefore, NMDARs composed of NR2A/B subunits, which confer a higher degree of Mg^{2+} block than NR2C/D subunits, contribute to LTP induction, and NMDARs composed of NR2C/D subunits contribute to LTD induction (Hrabetova, Serrano et al. 2000). These findings are particularly interesting with respect to the working model of this thesis, in which SK2 channels are hypothesized to modulate NMDAR activation through their effects on the NMDAR Mg^{2+} block. For example, it is possible that membrane repolarization via SK2 channel activation preferentially modulates the activity of NMDARs containing NR2A/B subunits, and therefore preferentially regulates mechanisms of LTP over LTD. In fact, in section IV of this thesis, SK2 overexpression attenuated LTP after 50 Hz high frequency stimulation, yet did not alter LTD after 1 Hz low frequency stimulation.

The SK channel- NMDAR feedback loop.

Initially, it was observed that that Ca^{2+} through NMDA receptors activates a Ca^{2+}-activated K^+ current in hippocampal neurons- providing some evidence that NMDA receptors and SK channels are colocalized in these neurons (Zorumski, Thio et al. 1989; Shah and Haylett 2002). Unfortunately, due to the lack of high-quality SK antibodies for

imaging, this has not been directly demonstrated. However, in two recent physiological studies, NMDAR/SK channel coupling has been observed in the hippocampus (Ngo-Anh, Bloodgood et al. 2005) and lateral amygdala (Faber, Delaney et al. 2005). In the former study, CA1 EPSPs were synaptically evoked and the NMDAR-mediated component of the EPSP was measured. With subthreshold synaptic activation, inward Ca^{2+} current is conducted primarily through NMDARs (Koester and Sakmann 1998; Yuste, Majewska et al. 1999; Kovalchuk, Eilers et al. 2000). Application of apamin to this preparation enhanced the amplitude of the EPSP in an NMDAR-dependent manner, suggesting that NMDARs and SK channels form a Ca^{2+}-mediated feedback loop- with Ca^{2+} through NMDARs activating SK channels, and in turn SK channels suppressing the NMDAR component of the EPSP via membrane repolarization. These data support the working model of this thesis nicely, which predicts that SK channels influence NMDAR activation through their membrane repolarization properties.

Figure 5.1

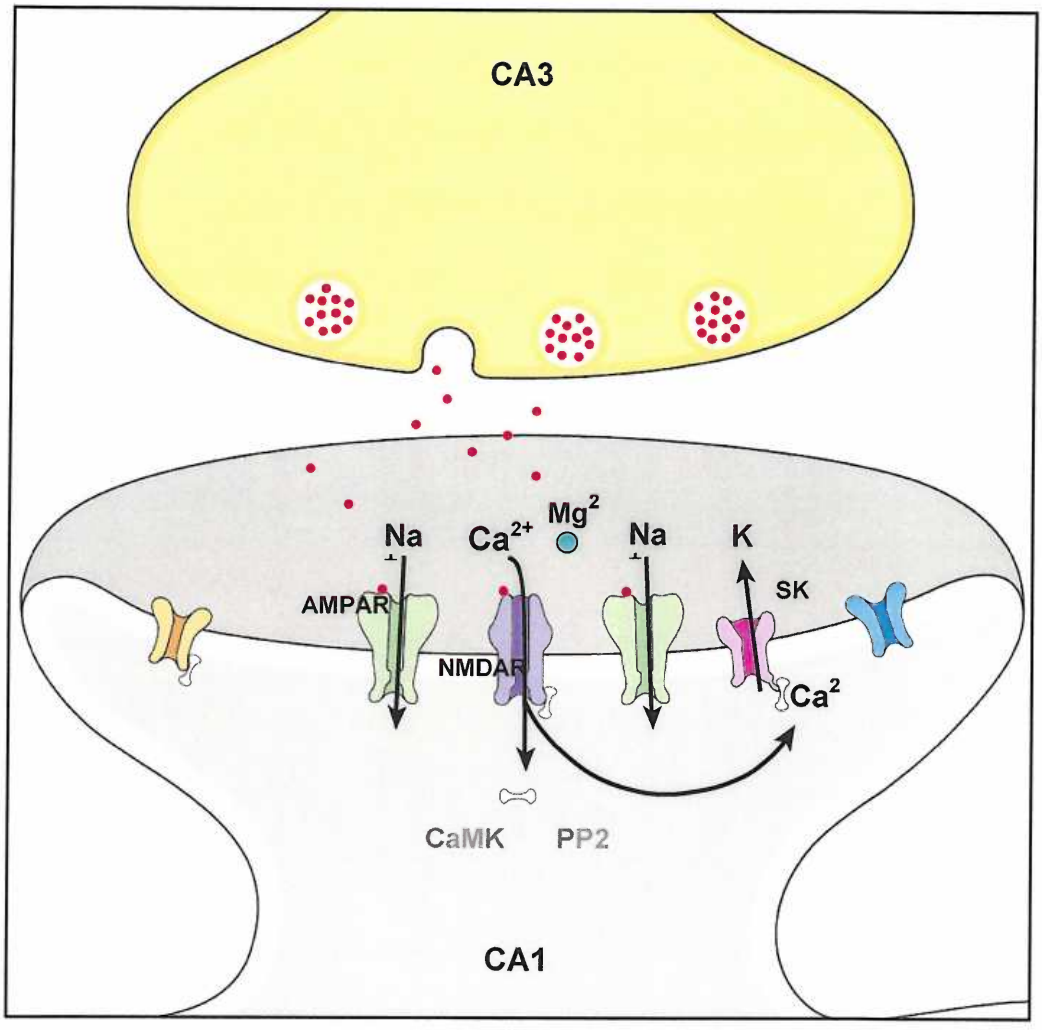


Figure 5.1 *Working model of SK2 regulation of hippocampal function*

In this model, SK2 channels located within the dendritic spine are activated by increases in intracellular Ca^{2+} through NMDARs. Outward K^+ current through SK2 channels repolarizes the postsynaptic membrane, reinstating the Mg^{2+} block of NMDARs. In this manner, SK2 channels modulate the activation of NMDARs, and therefore modulate NMDAR-dependent synaptic plasticity.

In addition, the spatial proximity of the synaptic Ca^{2+} source (possibly NMDARs) to SK channels has been estimated to be ~25-50 nm (Ngo-Anh, Bloodgood et al. 2005). This distance was calculated based on the differential effects of BAPTA and EGTA (two Ca^{2+} chelators with different Ca^{2+}-buffering time courses) on the apamin-induced increase in the EPSP. This study also demonstrated that in cultured CA1 neurons, overexpressed GFP-tagged SK2 channels 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 NMDARs).

Furthermore, in section IV of this thesis, similar experiments demonstrated that SK2 channels specifically contribute to the regulation of NMDAR activation via membrane repolarization. In this study, apamin's boosting effect of the EPSP was measured in CA1 neurons from WT and SK2+/T mice. SK2 overexpression resulted in a larger relative increase in the EPSP after apamin application, revealing that SK2 channels specifically regulate the NMDAR contribution to the EPSP. Together, these studies demonstrate that SK2 channels modulate NMDAR activation via membrane repolarization, which impacts the synaptically activated EPSP in the hippocampus. These data support the working model, providing a mechanism by which SK2 channels regulate hippocampal synaptic plasticity, learning and memory.

SK2 Channels and Intrinsic Excitability

As described above, the results of this thesis support a model in which SK2 channels regulate hippocampal function through their modulation of synaptic NMDAR activity in the hippocampus. However, it is also possible that SK2 channels also regulate hippocampal function through their effects on CA1 neuronal excitability. Recently, it has been suggested that modifications of intrinsic excitability can also mediate metaplastic processes involved in learning and memory systems (Saar and Barkai 2003; Zhang and Linden 2003). Hippocampal synaptic plasticity (specifically LTP and LTD) is a useful model for information storage based on its fundamental properties of input specificity and associativity. These synaptic modifications are eventually summated during synaptic integration, and subsequently it is the firing properties of the neuron that become the unit of information storage. Therefore, alterations in the intrinsic excitability of a neuron are capable of encoding information as well.

For example, increases in hippocampal neuronal excitability have been observed after trace eyeblink conditioning in rabbits (Moyer, Thompson et al. 1996). Trace eyeblink conditioning is a hippocampal-dependent paradigm that involves the presentation of an auditory cue (conditioned stimulus, CS) followed by an airpuff to the eye (unconditioned stimulus, US) resulting in reflexive blinking (unconditioned response). What is critical to the hippocampal-dependency of this task is the trace interval, which separates the CS and US, and requires that the animal learn the CS/US contingency. Once this contingency is learned, the animal will display its conditioned response; that is, when presented with the CS alone, the animal will blink in a timed

manner after the trace interval has passed. In this study, CA1 neurons recorded from conditioned rabbits exhibited a learning-induced increase in neuronal excitability, characterized by an increase in the number of spikes elicited from a depolarizing current injection (Moyer, Thompson et al. 1996). These increases in neuronal excitability were found to be specific to learning, since they were not observed in pseudo-conditioned animals or poor learners.

Importantly, the increases in intrinsic excitability of CA1 neurons from this study were accompanied by a reduction in the post-burst afterhyperpolarization (AHP). Specifically, it is the sAHP, which is mediated by a local Ca^{2+}-dependent K^+ conductance (Andreasen and Lambert 1995), that is reduced in these neurons, resulting in decreased spike-frequency adaptation and enhanced cell excitability (Sanchez-Andres and Alkon 1991). Similar reductions in CA1 post-burst AHPs have also been observed in rats trained in an olfactory-discrimination task (Zelcer, Cohen 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. Therefore, these data support the hypothesis that intrinsic excitability could serve as a metaplastic function in the hippocampus. Furthermore, because SK channels contribute to the mAHP in CA1 neurons (Stocker, Krause et al. 1999) it is possible that SK channels influence hippocampal metaplasticity (and therefore behavior) through their effects on intrinsic excitability.

There is still much to learn about how alterations in intrinsic excitability may relate to hippocampal synaptic plasticity. One possibility is that changes in intrinsic excitability alter the effectiveness of synaptic plasticity (Abraham and Bear 1996). For example, enhanced cell excitability after the activation of metabotropic glutamate receptors (mGluRs) primes subsequent synaptic LTP (Cohen and Abraham 1996). Another possibility is that properties of cell excitability themselves are long-term and convey metaplastic information. For example, long-term potentiation of intrinsic excitability (LTP-IE) involves a long-term (25-60 min) enhancement in the number of action potentials elicited with a given stimulation (via either current injection or synaptically evoked EPSPs). In pyramidal neurons of the hippocampus and layer V of the cortex, activation of group I mGluRs produces LTP-IE (Cohen and Abraham 1996; Ireland and Abraham 2002; Sourdet, Russier et al. 2003)[26]. LTP-IE has also been elicited with bursts of synaptic stimulation (10 Hz) in the presence of the ionotropic receptor antagonists kynurenate and picrotoxin (Sourdet, Russier et al. 2003).

Furthermore, LTP-IE has been associated with a reduction in both the sAHP and mAHP (Ireland and Abraham 2002; Sourdet, Russier et al. 2003), again implicating a mechanism whereby SK channels may influence hippocampal metaplasticity. In cortical neurons, blockade of SK channels with apamin enhanced the temporal precision of action potential firing similarly to that seen after the induction of LTP-IE, and activation of SK channels with EBIO reduced spike train precision (Sourdet, Russier et al. 2003), suggesting that SK channel regulation of excitability may contribute to the mechanisms underlying LTP-IE. However, in this study SK channel blockade with apamin reduced

[26]It is important to note here that LTP-IE and synaptic LTP priming elicited by mGluR activation are mediated by separate pathways, since particular mGluR antagonists (U-73122, chelerythrine) that block LTP priming do not affect LTP-IE. (Ireland and Abraham 2002)

the magnitude of LTP-IE, so it is unclear if the effects of SK channels on LTP-IE are related to the observed reduction in the mAHP.

While the studies from section IV of this thesis clearly demonstrate an effect of SK2 channel overexpression on hippocampal synaptic plasticity, results from the current literature also suggest the possibility that SK2 channels could modulate hippocampal metaplasticity and/or hippocampal-dependent behaviors through their regulation of the intrinsic excitability of CA1 neurons. Results from this thesis propose a clear mechanism by which SK2 channels regulate synaptic plasticity through their repolarization of the spine membrane and the subsequent modulation of NMDAR activation. This model is supported by the current literature, yet it remains possible that SK2 channels maintain parallel functions, and also play an important role in hippocampal processes mediated by properties of intrinsic excitability.

Summary and conclusions

Together, findings from this thesis demonstrate that SK2 channels play an important role in the regulation of hippocampal function, including declarative-like learning and memory processes. First, results from section II demonstrate that the long-delay object recognition paradigm is a hippocampal-dependent task. These results are significant because they validate the use of the object recognition task for the examination of mouse models of declarative memory. Next, results from section III of this thesis support previous findings that SK channels regulate hippocampal-dependent learning and memory processes. These results are significant because they demonstrate

that SK channels specifically modulate hippocampal memory *encoding* processes in mice. Furthermore, this is the first study to examine the specific role of *hippocampal* SK channels in learning and memory processes using intra-hippocampal microinjections of apamin. Lastly, results from section IV of this thesis demonstrate that the SK2 channel subtype specifically modulates hippocampal synaptic plasticity, learning, and memory. These studies are the first to examine the effects of SK2-specific overexpression on hippocampal function using a transgenic mouse model. Results from these studies indicate that SK2 channels regulate hippocampal function through the postsynaptic modulation of NMDAR activation. These findings are consistent with a model suggesting that SK2 channels are located in close proximity to NMDARs within the CA1 dendritic spine, forming a Ca^{2+}-mediated feedback loop.

References

Abraham, W. C. and M. F. Bear (1996). "Metaplasticity: the plasticity of synaptic plasticity." Trends Neurosci **19**(4): 126-30.

Aggleton, J. P., S. Keen, E. C. Warburton and T. J. Bussey (1997). "Extensive cytotoxic lesions involving both the rhinal cortices and area TE impair recognition but spare spatial alternation in the rat." Brain Res Bull **43**(3): 279-87.

Alkondon, M., E. F. Pereira, C. T. Barbosa and E. X. Albuquerque (1997). "Neuronal nicotinic acetylcholine receptor activation modulates gamma-aminobutyric acid release from CA1 neurons of rat hippocampal slices." J Pharmacol Exp Ther **283**(3): 1396-411.

Anagnostaras, S. G., S. Maren and M. S. Fanselow (1999). "Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination." J Neurosci **19**(3): 1106-14.

Anderson, P., T. V. Bliss and K. K. Skrede (1971). "Lamellar organization of hippocampal pathways." Exp Brain Res **13**(2): 222-38.

Andreasen, M. and J. D. Lambert (1995). "The excitability of CA1 pyramidal cell dendrites is modulated by a local Ca(2+)-dependent K(+)-conductance." Brain Res **698**(1-2): 193-203.

Aniksztejn, L. and Y. Ben-Ari (1991). "Novel form of long-term potentiation produced by a K+ channel blocker in the hippocampus." Nature **349**(6304): 67-9.

Artola, A. and W. Singer (1993). "Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation." Trends Neurosci **16**(11): 480-7.

Baker, K. B. and J. J. Kim (2002). "Effects of Stress and Hippocampal NMDA Receptor Antagonism on Recognition Memory in Rats." Learn Mem **9**(2): 58-65.

Barco, A., J. M. Alarcon and E. R. Kandel (2002). "Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture." Cell **108**(5): 689-703.

Bardgett, M. E., R. Boeckman, D. Krochmal, H. Fernando, R. Ahrens and J. G. Csernansky (2003). "NMDA receptor blockade and hippocampal neuronal loss impair fear conditioning and position habit reversal in C57Bl/6 mice." Brain Res Bull **60**(1-2): 131-42.

Barrionuevo, G., F. Schottler and G. Lynch (1980). "The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus." Life Sci **27**(24): 2385-91.

Baxter, M. G. and E. A. Murray (2001). "Opposite relationship of hippocampal and rhinal cortex damage to delayed nonmatching-to-sample deficits in monkeys." Hippocampus **11**(1): 61-71.

Beason-Held, L. L., D. L. Rosene, R. J. Killiany and M. B. Moss (1999). "Hippocampal formation lesions produce memory impairment in the rhesus monkey." Hippocampus **9**(5): 562-74.

Beattie, E. C., R. C. Carroll, X. Yu, W. Morishita, H. Yasuda, M. von Zastrow and R. C. Malenka (2000). "Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD." Nat Neurosci **3**(12): 1291-300.

Behnisch, T. and K. G. Reymann (1998). "Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro." Neurosci Lett **253**(2): 91-4.

Ben-Ari, Y., L. Aniksztejn and P. Bregestovski (1992). "Protein kinase C modulation of NMDA currents: an important link for LTP induction." Trends Neurosci **15**(9): 333-9.

Ben-Ari, Y., K. Krnjevic, R. J. Reiffenstein and W. Reinhardt (1981). "Inhibitory conductance changes and action of gamma-aminobutyrate in rat hippocampus." Neuroscience **6**(12): 2445-63.

Benke, T. A., A. Luthi, J. T. Isaac and G. L. Collingridge (1998). "Modulation of AMPA receptor unitary conductance by synaptic activity." Nature **393**(6687): 793-7.

Benton, D. C., A. S. Monaghan, R. Hosseini, P. K. Bahia, D. G. Haylett and G. W. Moss (2003). "Small conductance Ca2+-activated K+ channels formed by the expression of rat SK1 and SK2 genes in HEK 293 cells." J Physiol **553**(Pt 1): 13-9.

Bienenstock, E. L., L. N. Cooper and P. W. Munro (1982). "Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex." J Neurosci **2**(1): 32-48.

Blanchard, R. J. and D. C. Blanchard (1969). "Crouching as an index of fear." J Comp Physiol Psychol **67**(3): 370-5.

Blank, T., I. Nijholt, M. J. Kye, J. Radulovic and J. Spiess (2003). "Small-conductance, Ca2+-activated K+ channel SK3 generates age-related memory and LTP deficits." Nat Neurosci **6**(9): 911-2.

Blatz, A. L. and K. L. Magleby (1986). "Single apamin-blocked Ca-activated K^+ channels of small conductance in cultured rat skeletal muscle." Nature **323**(6090): 718-20.

Bliss, T. V. and G. L. Collingridge (1993). "A synaptic model of memory: long-term potentiation in the hippocampus." Nature **361**(6407): 31-9.

Blum, S., A. N. Moore, F. Adams and P. K. Dash (1999). "A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory." J Neurosci **19**(9): 3535-44.

Bond, C. T., P. S. Herson, T. Strassmaier, R. S. Hammond, R. W. Stackman, J. Maylie and J. P. Adelman (2004). "Small conductance Ca2+-activated K+ channel knock-out mice reveal the identity of calcium-dependent afterhyperpolarization currents." J Neurosci **24**(23): 5301-5306.

Bond, C. T., R. Sprengel, J. M. Bissonnette, W. A. Kaufmann, D. Pribnow, T. Neelands, T. Storck, M. Baetscher, J. Jerecic, J. Maylie, H. G. Knaus, P. H. Seeburg and J. P. Adelman (2000). "Respiration and parturition affected by conditional overexpression of the Ca^{2+}-activated K^+ channel subunit, SK3." Science **289**(5486): 1942-6.

Bourtchuladze, R., B. Frenguelli, J. Blendy, D. Cioffi, G. Schutz and A. J. Silva (1994). "Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein." Cell **79**(1): 59-68.

Brown, M. W. and J. P. Aggleton (2001). "Recognition memory: what are the roles of the perirhinal cortex and hippocampus?" Nat Rev Neurosci **2**(1): 51-61.

Bussey, T. J., J. Duck, J. L. Muir and J. P. Aggleton (2000). "Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of

the perirhinal and postrhinal cortices in the rat." Behav Brain Res **111**(1-2): 187-202.
Cahill, L., R. Babinsky, H. J. Markowitsch and J. L. McGaugh (1995). "The amygdala and emotional memory." Nature **377**(6547): 295-6.
Cahill, L., J. L. McGaugh and N. M. Weinberger (2001). "The neurobiology of learning and memory: some reminders to remember." Trends Neurosci **24**(10): 578-81.
Cai, X., C. W. Liang, S. Muralidharan, J. P. Kao, C. M. Tang and S. M. Thompson (2004). "Unique roles of SK and Kv4.2 potassium channels in dendritic integration." Neuron **44**(2): 351-64.
Cajal, S. R. y. (1911). Histologie du SyststemeNerveux de l'Homme et des Vertebres, Vol.II. Paris, Maloine.
Carroll, R. C., D. V. Lissin, M. von Zastrow, R. A. Nicoll and R. C. Malenka (1999). "Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures." Nat Neurosci **2**(5): 454-60.
Cassel, J. C., E. Duconseille, H. Jeltsch and B. Will (1997). "The fimbria-fornix/cingular bundle pathways: a review of neurochemical and behavioural approaches using lesions and transplantation techniques." Prog Neurobiol **51**(6): 663-716.
Castellani, G. C., E. M. Quinlan, L. N. Cooper and H. Z. Shouval (2001). "A biophysical model of bidirectional synaptic plasticity: dependence on AMPA and NMDA receptors." Proc Natl Acad Sci U S A **98**(22): 12772-7.
Cave, C. B. and L. R. Squire (1991). "Equivalent impairment of spatial and nonspatial memory following damage to the human hippocampus." Hippocampus **1**(3): 329-40.
Chen, C., J. J. Kim, R. F. Thompson and S. Tonegawa (1996). "Hippocampal lesions impair contextual fear conditioning in two strains of mice." Behav Neurosci **110**(5): 1177-80.
Cho, Y. H., E. Friedman and A. J. Silva (1999). "Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice." Behav Brain Res **98**(1): 77-87.
Cingolani, L. A., M. Gymnopoulos, A. Boccaccio, M. Stocker and P. Pedarzani (2002). "Developmental regulation of small-conductance Ca2+-activated K+ channel expression and function in rat Purkinje neurons." J Neurosci **22**(11): 4456-67.
Clark, R. E., S. M. Zola and L. R. Squire (2000). "Impaired recognition memory in rats after damage to the hippocampus." J Neurosci **20**(23): 8853-60.
Clusmann, H., R. Nitsch and U. Heinemann (1994). "Long lasting functional alterations in the rat dentate gyrus following entorhinal cortex lesion: a current source density analysis." Neuroscience **61**(4): 805-15.
Cohen, A. S. and W. C. Abraham (1996). "Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors." J Neurophysiol **76**(2): 953-62.
Colbert, C. M. and D. Johnston (1998). "Protein kinase C activation decreases activity-dependent attenuation of dendritic Na+ current in hippocampal CA1 pyramidal neurons." J Neurophysiol **79**(1): 491-5.
Cole, A. E. and R. A. Nicoll (1984). "The pharmacology of cholinergic excitatory responses in hippocampal pyramidal cells." Brain Res **305**(2): 283-90.

Collingridge, G. L., S. J. Kehl and H. McLennan (1983). "Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus." J Physiol **334**: 33-46.

Corcoran, K. A. and S. Maren (2001). "Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction." J Neurosci **21**(5): 1720-6.

Cummings, J. A., R. M. Mulkey, R. A. Nicoll and R. C. Malenka (1996). "Ca2+ signaling requirements for long-term depression in the hippocampus." Neuron **16**(4): 825-33.

Daum, I., M. M. Schugens, H. Ackermann, W. Lutzenberger, J. Dichgans and N. Birbaumer (1993). "Classical conditioning after cerebellar lesions in humans." Behav Neurosci **107**(5): 748-56.

Davis, M. (1994). "The role of the amygdala in emotional learning." Int Rev Neurobiol **36**: 225-66.

Davis, S., S. P. Butcher and R. G. Morris (1992). "The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro." J Neurosci **12**(1): 21-34.

Deisseroth, K., H. Bito and R. W. Tsien (1996). "Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity." Neuron **16**(1): 89-101.

Derkach, V., A. Barria and T. R. Soderling (1999). "Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors." Proc Natl Acad Sci U S A **96**(6): 3269-74.

Deschaux, O. and J. C. Bizot (1997). "Effect of apamin, a selective blocker of Ca2+-activated K+-channel, on habituation and passive avoidance responses in rats." Neurosci Lett **227**(1): 57-60.

Deschaux, O., J. C. Bizot and M. Goyffon (1997). "Apamin improves learning in an object recognition task in rats." Neurosci Lett **222**(3): 159-62.

Disterhoft, J. F., D. T. Golden, H. L. Read, D. A. Coulter and D. L. Alkon (1988). "AHP reductions in rabbit hippocampal neurons during conditioning correlate with acquisition of the learned response." Brain Res **462**(1): 118-25.

Dix, S. L. and J. P. Aggleton (1999). "Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition." Behav Brain Res **99**(2): 191-200.

Dodart, J. C., C. Mathis and A. Ungerer (1997). "Scopolamine-induced deficits in a two-trial object recognition task in mice." Neuroreport **8**(5): 1173-8.

Dudek, S. M. and M. F. Bear (1992). "Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade." Proc Natl Acad Sci U S A **89**(10): 4363-7.

Dusek, J. A. and H. Eichenbaum (1998). "The hippocampus and transverse patterning guided by olfactory cues." Behav Neurosci **112**(4): 762-71.

Eichenbaum, H. (1997). "Declarative memory: insights from cognitive neurobiology." Annu Rev Psychol **48**: 547-72.

Eichenbaum, H. (1999). "The hippocampus and mechanisms of declarative memory." Behav Brain Res **103**(2): 123-33.

Eichenbaum, H. (2000). "A cortical-hippocampal system for declarative memory." Nat Rev Neurosci **1**(1): 41-50.

Eichenbaum, H., A. Fagan and N. J. Cohen (1986). "Normal olfactory discrimination learning set and facilitation of reversal learning after medial-temporal damage in rats: implications for an account of preserved learning abilities in amnesia." J Neurosci **6**(7): 1876-84.

Eichenbaum, H., A. Fagan, P. Mathews and N. J. Cohen (1988). "Hippocampal system dysfunction and odor discrimination learning in rats: impairment or facilitation depending on representational demands." Behav Neurosci **102**(3): 331-9.

Eichenbaum, H., P. Mathews and N. J. Cohen (1989). "Further studies of hippocampal representation during odor discrimination learning." Behav Neurosci **103**(6): 1207-16.

Eichenbaum, H., C. Stewart and R. G. M. Morris (1990). "Hippocampal representation in place learning." Journal of Neuroscience **10**(11): 3531-3542.

Ennaceur, A. and J. Delacour (1988). "A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data." Behav Brain Res **31**(1): 47-59.

Ennaceur, A., N. Neave and J. P. Aggleton (1997). "Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix." Exp Brain Res **113**(3): 509-19.

Faber, E. S., A. J. Delaney and P. Sah (2005). "SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala." Nat Neurosci **8**(5): 635-41.

Faber, E. S., A. J. Delaney and P. Sah (2005). "SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala." Nature Neurosci **8**: 635-641.

Faber, E. S. and P. Sah (2002). "Physiological role of calcium-activated potassium currents in the rat lateral amygdala." J Neurosci **22**(5): 1618-28.

Forsythe, I. D. and G. L. Westbrook (1988). "Slow excitatory postsynaptic currents mediated by N-methyl-D-aspartate receptors on cultured mouse central neurones." J Physiol **396**: 515-33.

Fortin, N. J., K. L. Agster and H. B. Eichenbaum (2002). "Critical role of the hippocampus in memory for sequences of events." Nat Neurosci **5**(5): 458-62.

Fortin, N. J., S. P. Wright and H. Eichenbaum (2004). "Recollection-like memory retrieval in rats is dependent on the hippocampus." Nature **431**(7005): 188-91.

Frey, U., M. Krug, K. G. Reymann and H. Matthies (1988). "Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro." Brain Res **452**(1-2): 57-65.

Gaffan, E. A., M. J. Eacott and E. L. Simpson (2000). "Perirhinal cortex ablation in rats selectively impairs object identification in a simultaneous visual comparison task." Behav Neurosci **114**(1): 18-31.

Gallagher, M., R. Burwell and M. Burchinal (1993). "Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze." Behav Neurosci **107**(4): 618-26.

Gardos, G. (1958). "The function of calcium in the potassium permeability of human erythrocytes." Biochim Biophys Acta **30**(3): 653-4.

Gaskin, S., A. Tremblay and D. G. Mumby (2003). "Retrograde and anterograde object recognition in rats with hippocampal lesions." Hippocampus **13**(8): 962-9.

Ghelardini, C., N. Galeotti and A. Bartolini (1998). "Influence of potassium channel modulators on cognitive processes in mice." Br J Pharmacol **123**(6): 1079-84.

Gilbert, P. E. and R. P. Kesner (2003). "Recognition memory for complex visual discriminations is influenced by stimulus interference in rodents with perirhinal cortex damage." Learn Mem **10**(6): 525-30.

Gold, J. I. and M. F. Bear (1994). "A model of dendritic spine Ca2+ concentration exploring possible bases for a sliding synaptic modification threshold." Proc Natl Acad Sci U S A **91**(9): 3941-5.

Graf, P., L. R. Squire and G. Mandler (1984). "The information that amnesic patients do not forget." J Exp Psychol Learn Mem Cogn **10**(1): 164-78.

Gray, R., A. S. Rajan, K. A. Radcliffe, M. Yakehiro and J. A. Dani (1996). "Hippocampal synaptic transmission enhanced by low concentrations of nicotine." Nature **383**(6602): 713-6.

Grover, L. M. and T. J. Teyler (1990). "Two components of long-term potentiation induced by different patterns of afferent activation." Nature **347**(6292): 477-9.

Gu, N., K. Vervaeke, H. Hu and J. F. Storm (2005). "Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization (mAHP)and excitability control in CA1 hippocampal pyramidal cells." J Physiol.

Hallworth, N. E., C. J. Wilson and M. D. Bevan (2003). "Apamin-sensitive small conductance calcium-activated potassium channels, through their selective coupling to voltage-gated calcium channels, are critical determinants of the precision, pace, and pattern of action potential generation in rat subthalamic nucleus neurons in vitro." J Neurosci **23**(20): 7525-42.

Hammond, R. S., C. T. Bond, T. J. Ngo-Anh, J. P. Adelman, J. Maylie and R. W. Stackman (2005). "Small-conductance Ca2+-activated K+ channel type 2 (SK2) overexpression impairs hippocampal learning, memory, and synaptic plasticity." Nature Neurosci **Submitted**.

Hammond, R. S., L. E. Tull and R. W. Stackman (2004). "On the delay-dependent involvement of the hippocampus in object recognition memory." Neurobiol Learn Mem **82**(1): 26-34.

Hammond, R. S., L. E. Tull, T. Tzounopoulos and R. W. Stackman (2002). "The influence of hippocampal Ca2+-activated K+ (SK) channels in spatial and nonspatial memory encoding." Society for Neuroscience Abstracts **28**: 80.12.

Hebb, D. O. (1949). The Organization of Behaviour. New York, Wiley.

Heurteaux, C., C. Messier, C. Destrade and M. Lazdunski (1993). "Memory processing and apamin induce immediate early gene expression in mouse brain." Brain Res Mol Brain Res **18**(1-2): 17-22.

Hoffman, D. A., J. C. Magee, C. M. Colbert and D. Johnston (1997). "K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons." Nature **387**(6636): 869-75.

Hrabetova, S., P. Serrano, N. Blace, H. W. Tse, D. A. Skifter, D. E. Jane, D. T. Monaghan and T. C. Sacktor (2000). "Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction." J Neurosci **20**(12): RC81.

Ikonen, S. and P. Riekkinen, Jr. (1999). "Effects of apamin on memory processing of hippocampal-lesioned mice." Eur J Pharmacol **382**(3): 151-6.

Ikonen, S., B. Schmidt and P. Riekkinen, Jr. (1998). "Apamin improves spatial navigation in medial septal-lesioned mice." Eur J Pharmacol **347**(1): 13-21.

Ireland, D. R. and W. C. Abraham (2002). "Group I mGluRs increase excitability of hippocampal CA1 pyramidal neurons by a PLC-independent mechanism." J Neurophysiol **88**(1): 107-16.

Ishii, T. M., J. Maylie and J. P. Adelman (1997). "Determinants of apamin and d-tubocurarine block in SK potassium channels." J Biol Chem **272**(37): 23195-200.

Ishii, T. M., C. Silvia, B. Hirschberg, C. T. Bond, J. P. Adelman and J. Maylie (1997). "A human intermediate conductance calcium-activated potassium channel." Proc Natl Acad Sci U S A **94**(21): 11651-6.

Johnson, S. W. and V. Seutin (1997). "Bicuculline methiodide potentiates NMDA-dependent burst firing in rat dopamine neurons by blocking apamin-sensitive Ca2+-activated K+ currents." Neurosci Lett **231**(1): 13-6.

Joiner, W. J., L. Y. Wang, M. D. Tang and L. K. Kaczmarek (1997). "hSK4, a member of a novel subfamily of calcium-activated potassium channels." Proc Natl Acad Sci U S A **94**(20): 11013-8.

Kapur, A., M. F. Yeckel, R. Gray and D. Johnston (1998). "L-Type calcium channels are required for one form of hippocampal mossy fiber LTP." J Neurophysiol **79**(4): 2181-90.

Kawai, T. and M. Watanabe (1986). "Blockade of Ca-activated K conductance by apamin in rat sympathetic neurones." Br J Pharmacol **87**(1): 225-32.

Kelly, A., S. Laroche and S. Davis (2003). "Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory." J Neurosci **23**(12): 5354-60.

Kim, J. J. and M. S. Fanselow (1992). "Modality-specific retrograde amnesia of fear." Science **256**(5057): 675-7.

Kim, J. J., R. A. Rison and M. S. Fanselow (1993). "Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear." Behav Neurosci **107**(6): 1093-8.

Kinney, G. G., P. Patino, Y. Mermet-Bouvier, J. E. Starrett and V. K. Gribkoff (1999). "Cognition-enhancing drugs increase stimulated hippocampal theta rhythm amplitude in the urethane-anesthetized rat." J Pharmacol Exp Ther **291**(1): 99-106.

Klann, E., S. J. Chen and J. D. Sweatt (1993). "Mechanism of protein kinase C activation during the induction and maintenance of long-term potentiation probed using a selective peptide substrate." Proc Natl Acad Sci U S A **90**(18): 8337-41.

Knowlton, B. J., J. A. Mangels and L. R. Squire (1996). "A neostriatal habit learning system in humans." Science **273**(5280): 1399-402.

Koester, H. J. and B. Sakmann (1998). "Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials." Proc Natl Acad Sci U S A **95**(16): 9596-601.

Kohler, M., B. Hirschberg, C. T. Bond, J. M. Kinzie, N. V. Marrion, J. Maylie and J. P. Adelman (1996). "Small-conductance, calcium-activated potassium channels from mammalian brain." Science **273**(5282): 1709-14.

Kovalchuk, Y., J. Eilers, J. Lisman and A. Konnerth (2000). "NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons." J Neurosci **20**(5): 1791-9.

Kramar, E. A., B. Lin, C. Y. Lin, A. C. Arai, C. M. Gall and G. Lynch (2004). "A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor." J Neurosci **24**(22): 5151-61.

Lancaster, B. and R. A. Nicoll (1987). "Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones." J Physiol **389**: 187-203.

Larson, J., D. Wong and G. Lynch (1986). "Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation." Brain Res **368**(2): 347-50.

LeDoux, J. E. (1993). "Emotional memory systems in the brain." Behav Brain Res **58**(1-2): 69-79.

LeDoux, J. E. (2000). "Emotion circuits in the brain." Annu Rev Neurosci **23**: 155-84.

Lee, H. K., M. Barbarosie, K. Kameyama, M. F. Bear and R. L. Huganir (2000). "Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity." Nature **405**(6789): 955-9.

Lee, H. K., K. Kameyama, R. L. Huganir and M. F. Bear (1998). "NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus." Neuron **21**(5): 1151-62.

Lee, I. and R. P. Kesner (2003). "Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory." J Neurosci **23**(4): 1517-23.

Li, B. S., M. K. Sun, L. Zhang, S. Takahashi, W. Ma, L. Vinade, A. B. Kulkarni, R. O. Brady and H. C. Pant (2001). "Regulation of NMDA receptors by cyclin-dependent kinase-5." Proc Natl Acad Sci U S A **98**(22): 12742-7.

Liao, D., N. A. Hessler and R. Malinow (1995). "Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice." Nature **375**(6530): 400-4.

Lisman, J. (1989). "A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory." Proc Natl Acad Sci U S A **86**(23): 9574-8.

Lisman, J., H. Schulman and H. Cline (2002). "The molecular basis of CaMKII function in synaptic and behavioural memory." Nat Rev Neurosci **3**(3): 175-90.

Liu, P. and D. K. Bilkey (2001). "The effect of excitotoxic lesions centered on the hippocampus or perirhinal cortex in object recognition and spatial memory tasks." Behav Neurosci **115**(1): 94-111.

Logue, S. F., R. Paylor and J. M. Wehner (1997). "Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task." Behav Neurosci **111**(1): 104-13.

Lynch, G., J. Larson, S. Kelso, G. Barrionuevo and F. Schottler (1983). "Intracellular injections of EGTA block induction of hippocampal long-term potentiation." Nature **305**(5936): 719-21.

Lynch, G. S., T. Dunwiddie and V. Gribkoff (1977). "Heterosynaptic depression: a postsynaptic correlate of long-term potentiation." Nature **266**(5604): 737-9.

Malenka, R. C., J. A. Kauer, R. S. Zucker and R. A. Nicoll (1988). "Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission." Science **242**(4875): 81-4.

Malenka, R. C. and R. A. Nicoll (1993). "NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms." Trends Neurosci **16**(12): 521-7.

Malenka, R. C. and R. A. Nicoll (1999). "Long-term potentiation--a decade of progress?" Science **285**(5435): 1870-4.

Malinow, R. and R. C. Malenka (2002). "AMPA receptor trafficking and synaptic plasticity." Annu Rev Neurosci **25**: 103-26.

Maren, S., G. Aharonov and M. S. Fanselow (1997). "Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats." Behav Brain Res **88**(2): 261-74.

Maren, S. and M. S. Fanselow (1997). "Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats." Neurobiol Learn Mem **67**(2): 142-9.

Marty, A. (1981). "Ca-dependent K channels with large unitary conductance in chromaffin cell membranes." Nature **291**(5815): 497-500.

McDonald, R. J. and N. M. White (1994). "Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus." Behav Neural Biol **61**(3): 260-70.

McEchron, M. D., H. Bouwmeester, W. Tseng, C. Weiss and J. F. Disterhoft (1998). "Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat." Hippocampus **8**(6): 638-46.

McEchron, M. D., A. P. Weible and J. F. Disterhoft (2001). "Aging and learning-specific changes in single-neuron activity in CA1 hippocampus during rabbit trace eyeblink conditioning." J Neurophysiol **86**(4): 1839-57.

McNaughton, B. L. and C. A. Barnes (1977). "Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat." J Comp Neurol **175**(4): 439-54.

McNaughton, B. L., R. M. Douglas and G. V. Goddard (1978). "Synaptic enhancement in fascia dentata: cooperativity among coactive afferents." Brain Res **157**(2): 277-93.

McNish, K. A., J. C. Gewirtz and M. Davis (1997). "Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle." J Neurosci **17**(23): 9353-60.

Messier, C., C. Mourre, B. Bontempi, J. Sif, M. Lazdunski and C. Destrade (1991). "Effect of apamin, a toxin that inhibits Ca^{2+}-dependent K^{+} channels, on learning and memory processes." Brain Res **551**(1-2): 322-6.

Meunier, M., J. Bachevalier, M. Mishkin and E. A. Murray (1993). "Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys." J Neurosci **13**(12): 5418-32.

Milner, B. (1972). "Disorders of learning and memory after temporal lobe lesions in man." Clin Neurosurg **19**: 421-46.

Milner, B. and W. Penfield (1955). "The effect of hippocampal lesions on recent memory." Trans Am Neurol Assoc(80th Meeting): 42-8.

Milner, B., L. R. Squire and E. R. Kandel (1998). "Cognitive neuroscience and the study of memory." Neuron **20**(3): 445-68.

Mishkin, M. (1978). "Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus." Nature **273**(5660): 297-8.

Morris, R. G. (1989). "Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D- aspartate receptor antagonist AP5." J Neurosci **9**(9): 3040-57.

Morris, R. G., E. Anderson, G. S. Lynch and M. Baudry (1986). "Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5." Nature **319**(6056): 774-6.

Morris, R. G. M., P. Garrud, J. N. P. Rawlins and J. O'Keefe (1982). "Place navigation impaired in rats with hippocampal lesions." Nature **297**: 681-683.

Morris, R. G. M., F. Schenk, F. Tweedie and L. E. Jarrard (1990). "Ibotenate lesions of hippocampus and/or subiculum: dissociating components of allocentric spatial learning." Eur J Neurosci **2**: 1016-1028.

Moser, E. I., K. A. Krobert, M. B. Moser and R. G. Morris (1998). "Impaired spatial learning after saturation of long-term potentiation [see comments]." Science **281**(5385): 2038-42.

Moser, M. B., E. I. Moser, E. Forrest, P. Andersen and R. G. Morris (1995). "Spatial learning with a minislab in the dorsal hippocampus." Proc Natl Acad Sci U S A **92**(21): 9697-701.

Mourre, C., P. Cervera and M. Lazdunski (1987). "Autoradiographic analysis in rat brain of the postnatal ontogeny of voltage-dependent Na^+ channels, Ca^{2+}-dependent K^+ channels and slow Ca^{2+} channels identified as receptors for tetrodotoxin, apamin and (-)-desmethoxyverapamil." Brain Res **417**(1): 21-32.

Mourre, C., M. Hugues and M. Lazdunski (1986). "Quantitative autoradiographic mapping in rat brain of the receptor of apamin, a polypeptide toxin specific for one class of Ca2+-dependent K+ channels." Brain Res **382**(2): 239-49.

Moyer, J. R., Jr., L. T. Thompson and J. F. Disterhoft (1996). "Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner." J Neurosci **16**(17): 5536-46.

Mulkey, R. M., S. Endo, S. Shenolikar and R. C. Malenka (1994). "Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression." Nature **369**(6480): 486-8.

Mulkey, R. M., C. E. Herron and R. C. Malenka (1993). "An essential role for protein phosphatases in hippocampal long-term depression." Science **261**(5124): 1051-5.

Mulkey, R. M. and R. C. Malenka (1992). "Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus." Neuron **9**(5): 967-75.

Mumby, D. G. (2001). "Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats." Behav Brain Res **127**(1-2): 159-81.

Mumby, D. G., R. S. Astur, M. P. Weisend and R. J. Sutherland (1999). "Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations." Behav Brain Res **106**(1-2): 97-107.

Mumby, D. G., S. Gaskin, M. J. Glenn, T. E. Schramek and H. Lehmann (2002). "Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts." Learn Mem **9**(2): 49-57.

Murray, E. A., T. J. Bussey, R. R. Hampton and L. M. Saksida (2000). "The parahippocampal region and object identification." Ann N Y Acad Sci **911**: 166-74.

Murray, E. A. and M. Mishkin (1998). "Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus." J Neurosci **18**(16): 6568-82.

Murray, E. A. and B. J. Richmond (2001). "Role of perirhinal cortex in object perception, memory, and associations." Curr Opin Neurobiol **11**(2): 188-93.

Nevian, T. and B. Sakmann (2004). "Single spine Ca2+ signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex." J Neurosci **24**(7): 1689-99.

Newberry, N. R. and R. A. Nicoll (1984). "Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells." Nature **308**(5958): 450-2.

Ngo-Anh, T. J., B. L. Bloodgood, M. Lin, B. L. Sabatini, J. Maylie and J. Adelman (2005). "SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines." Nat Neurosci.

Ngo-Anh, T. J., B. L. Bloodgood, M. Lin, B. L. Sabatini, J. Maylie and J. P. Adelman (2005). "SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines." Nat Neurosci **8**(5): 642-9.

Nordholm, A. F., J. K. Thompson, C. Dersarkissian and R. F. Thompson (1993). "Lidocaine infusion in a critical region of cerebellum completely prevents learning of the conditioned eyeblink response." Behav Neurosci **107**(5): 882-6.

Obermair, G. J., W. A. Kaufmann, H. G. Knaus and B. E. Flucher (2003). "The small conductance Ca2+-activated K+ channel SK3 is localized in nerve terminals of excitatory synapses of cultured mouse hippocampal neurons." Eur J Neurosci **17**(4): 721-31.

Oh, M. M., A. G. Kuo, W. W. Wu, E. A. Sametsky and J. F. Disterhoft (2003). "Watermaze learning enhances excitability of CA1 pyramidal neurons." J Neurophysiol **90**(4): 2171-9.

Oh, M. M., J. M. Power, L. T. Thompson and J. F. Disterhoft (2000). "Apamin increases excitability of CA1 hippocampal pyramidal neurons." Neuroscience Research Communications **27**(2): 135-142.

O'Keefe, J. (1976). "Place units in the hippocampus of the freely moving rat." Exp Neurol **51**: 78-109.

O'Keefe, J. and L. Nadel (1978). The Hippocampus as a Cognitive Map. UK, Clarendon Press, Oxford.

Olton, D. S., J. A. Walker and F. H. Gage (1978). "Hippocampal connections and spatial discrimination." Brain Res **139**(2): 295-308.

Otmakhova, N. A. and J. E. Lisman (2004). "Contribution of Ih and GABAB to synaptically induced afterhyperpolarizations in CA1: a brake on the NMDA response." J Neurophysiol **92**(4): 2027-39.

Packard, M. G., R. Hirsh and N. M. White (1989). "Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems." J Neurosci **9**(5): 1465-72.

Paxinos, G. and K. B. J. Franklin (2001). The mouse brain in stereotaxic coordinates. San Diego, CA, Academic Press.

Paylor, R., R. Tracy, J. Wehner and J. W. Rudy (1994). "DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning." Behav Neurosci **108**(4): 810-7.

Pedarzani, P., J. Mosbacher, A. Rivard, L. A. Cingolani, D. Oliver, M. Stocker, J. P. Adelman and B. Fakler (2001). "Control of electrical activity in central neurons by modulating the gating of small conductance Ca^{2+}-activated K^+ channels." J Biol Chem **276**(13): 9762-9.

Pettit, D. L., S. Perlman and R. Malinow (1994). "Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons." Science **266**(5192): 1881-5.

Phillips, R. G. and J. E. LeDoux (1992). "Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning." Behav Neurosci **106**(2): 274-85.

Pinsker, H., I. Kupfermann, V. Castellucci and E. Kandel (1970). "Habituation and dishabituation of the gill-withdrawal reflex in Aplysia." Science **167**(926): 1740-2.

Pinsker, H. M., W. A. Hening, T. J. Carew and E. R. Kandel (1973). "Long-term sensitization of a defensive withdrawal reflex in Aplysia." Science **182**(116): 1039-42.

Poorheidari, G., K. J. Stanhope and J. A. Pratt (1998). "Effects of the potassium channel blockers, apamin and 4-aminopyridine, on scopolamine-induced deficits in the delayed matching to position task in rats: a comparison with the cholinesterase inhibitor E2020." Psychopharmacology (Berl) **135**(3): 242-55.

Puma, C., C. Baudoin and J. C. Bizot (1998). "Effects of intraseptal infusions of N-methyl-D-aspartate receptor ligands on memory in an object recognition task in rats." Neurosci Lett **244**(2): 97-100.

Puma, C. and J. C. Bizot (1998). "Intraseptal infusions of a low dose of AP5, a NMDA receptor antagonist, improves memory in an object recognition task in rats." Neurosci Lett **248**(3): 183-6.

Rampon, C., Y. P. Tang, J. Goodhouse, E. Shimizu, M. Kyin and J. Z. Tsien (2000). "Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice." Nat Neurosci **3**(3): 238-44.

Raymond, L. A., W. G. Tingley, C. D. Blackstone, K. W. Roche and R. L. Huganir (1994). "Glutamate receptor modulation by protein phosphorylation." J Physiol Paris **88**(3): 181-92.

Reed, J. M. and L. R. Squire (1997). "Impaired recognition memory in patients with lesions limited to the hippocampal formation." Behav Neurosci **111**(4): 667-75.

Regehr, W. G. and D. W. Tank (1990). "Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites." Nature **345**(6278): 807-10.

Rempel-Clower, N. L., S. M. Zola, L. R. Squire and D. G. Amaral (1996). "Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation." J Neurosci **16**(16): 5233-55.

Richmond, M. A., B. K. Yee, B. Pouzet, L. Veenman, J. N. Rawlins, J. Feldon and D. M. Bannerman (1999). "Dissociating context and space within the hippocampus:

effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning." Behav Neurosci **113**(6): 1189-203.

Riedel, G., J. Micheau, A. G. Lam, E. Roloff, S. J. Martin, H. Bridge, L. Hoz, B. Poeschel, J. McCulloch and R. G. Morris (1999). "Reversible neural inactivation reveals hippocampal participation in several memory processes." Nat Neurosci **2**(10): 898-905.

Rogan, M. T. and J. E. LeDoux (1995). "LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit." Neuron **15**(1): 127-36.

Rogan, M. T., U. V. Staubli and J. E. LeDoux (1997). "Fear conditioning induces associative long-term potentiation in the amygdala." Nature **390**(6660): 604-7.

Romey, G. and M. Lazdunski (1984). "The coexistence in rat muscle cells of two distinct classes of Ca2+-dependent K+ channels with different pharmacological properties and different physiological functions." Biochem Biophys Res Commun **118**(2): 669-74.

Ropert, N. and N. Guy (1991). "Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus in vitro." J Physiol **441**: 121-36.

Rudy, J. W., R. M. Barrientos and R. C. O'Reilly (2002). "Hippocampal formation supports conditioning to memory of a context." Behav Neurosci **116**(4): 530-8.

Rudy, J. W., N. C. Huff and P. Matus-Amat (2004). "Understanding contextual fear conditioning: insights from a two-process model." Neurosci Biobehav Rev **28**(7): 675-85.

Saar, D. and E. Barkai (2003). "Long-term modifications in intrinsic neuronal properties and rule learning in rats." Eur J Neurosci **17**(12): 2727-34.

Sacktor, T. C., P. Osten, H. Valsamis, X. Jiang, M. U. Naik and E. Sublette (1993). "Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation." Proc Natl Acad Sci U S A **90**(18): 8342-6.

Sah, P. and E. S. Faber (2002). "Channels underlying neuronal calcium-activated potassium currents." Prog Neurobiol **66**(5): 345-53.

Sailer, C. A., H. Hu, W. A. Kaufmann, M. Trieb, C. Schwarzer, J. F. Storm and H. G. Knaus (2002). "Regional differences in distribution and functional expression of small-conductance Ca2+-activated K+ channels in rat brain." J Neurosci **22**(22): 9698-707.

Sailer, C. A., W. A. Kaufmann, J. Marksteiner and H. G. Knaus (2004). "Comparative immunohistochemical distribution of three small-conductance Ca2+-activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain." Mol Cell Neurosci **26**(3): 458-69.

Sanchez-Andres, J. V. and D. L. Alkon (1991). "Voltage-clamp analysis of the effects of classical conditioning on the hippocampus." J Neurophysiol **65**(4): 796-807.

Sandkuhler, J., B. Maisch and M. Zimmermann (1987). "The use of local anaesthetic microinjections to identify central pathways: a quantitative evaluation of the time course and extent of the neuronal block." Exp Brain Res **68**(1): 168-78.

Scoville, W. B. and B. Milner (1957). "Loss of recent memory after bilateral hippocampal lesions." Journal of Neurology, Neurosurgery and Psychiatry **20**: 11-21.

Selcher, J. C., C. M. Atkins, J. M. Trzaskos, R. Paylor and J. D. Sweatt (1999). "A necessity for MAP kinase activation in mammalian spatial learning." Learn Mem **6**(5): 478-90.

Shah, M. and D. G. Haylett (2000). "The pharmacology of hSK1 Ca2+-activated K+ channels expressed in mammalian cell lines." Br J Pharmacol **129**(4): 627-30.

Shah, M. M. and D. G. Haylett (2002). "K^+ currents generated by NMDA receptor activation in rat hippocampal pyramidal neurons." J Neurophysiol **87**: 2983-89.

Shakkottai, V. G., I. Regaya, H. Wulff, Z. Fajloun, H. Tomita, M. Fathallah, M. D. Cahalan, J. J. Gargus, J. M. Sabatier and K. G. Chandy (2001). "Design and characterization of a highly selective peptide inhibitor of the small conductance calcium-activated K+ channel, SkCa2." J Biol Chem **276**(46): 43145-51.

Shao, L. R., R. Halvorsrud, L. Borg-Graham and J. F. Storm (1999). "The role of BK-type Ca2+-dependent K+ channels in spike broadening during repetitive firing in rat hippocampal pyramidal cells." J Physiol **521 Pt 1**: 135-46.

Shi, S. H., Y. Hayashi, R. S. Petralia, S. H. Zaman, R. J. Wenthold, K. Svoboda and R. Malinow (1999). "Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation." Science **284**(5421): 1811-6.

Shors, T. J. and L. D. Matzel (1997). "Long-term potentiation: what's learning got to do with it?" Behav Brain Sci **20**(4): 597-655.

Sik, A., P. van Nieuwehuyzen, J. Prickaerts and A. Blokland (2003). "Performance of different mouse strains in an object recognition task." Behav Brain Res **147**(1-2): 49-54.

Silva, A. J., R. Paylor, J. M. Wehner and S. Tonegawa (1992). "Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice [see comments]." Science **257**(5067): 206-11.

Silva, A. J., Y. Wang, R. Paylor, J. M. Wehner, C. F. Stevens and S. Tonegawa (1992). "Alpha calcium/calmodulin kinase II mutant mice: deficient long-term potentiation and impaired spatial learning." Cold Spring Harb Symp Quant Biol **57**: 527-39.

Sobczyk, A., V. Scheuss and K. Svoboda (2005). "NMDA receptor subunit-dependent [Ca2+] signaling in individual hippocampal dendritic spines." J Neurosci **25**(26): 6037-46.

Soderling, T. R. and V. A. Derkach (2000). "Postsynaptic protein phosphorylation and LTP." Trends Neurosci **23**(2): 75-80.

Sourdet, V., M. Russier, G. Daoudal, N. Ankri and D. Debanne (2003). "Long-term enhancement of neuronal excitability and temporal fidelity mediated by metabotropic glutamate receptor subtype 5." J Neurosci **23**(32): 10238-48.

Squire, L. R. (1992). "Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans." Psychol Rev **99**(2): 195-231.

Squire, L. R., D. G. Amaral and G. A. Press (1990). "Magnetic resonance imaging of the hippocampal formation and mammillary nuclei distinguish medial temporal lobe and diencephalic amnesia." J Neurosci **10**(9): 3106-17.

Squire, L. R., C. E. Stark and R. E. Clark (2004). "The medial temporal lobe." Annu Rev Neurosci **27**: 279-306.

Squire, L. R. and S. M. Zola (1996). "Structure and function of declarative and nondeclarative memory systems." Proc Natl Acad Sci U S A **93**(24): 13515-22.

Squire, L. R. and S. Zola-Morgan (1991). "The medial temporal lobe memory system." Science **253**(5026): 1380-6.

Stackman, R. W., R. S. Hammond, E. Linardatos, A. Gerlach, J. Maylie, J. P. Adelman and T. Tzounopoulos (2002). "Small conductance Ca^{2+}-activated K^+ channels modulate synaptic plasticity and memory encoding." J Neurosci **22**(23): 10163-71.

Stocker, M., K. Hirzel, D. D'Hoedt and P. Pedarzani (2004). "Matching molecules to function: neuronal Ca2+-activated K+ channels and afterhyperpolarizations." Toxicon **43**(8): 933-49.

Stocker, M., M. Krause and P. Pedarzani (1999). "An apamin-sensitive Ca^{2+}-activated K^+ current in hippocampal pyramidal neurons." Proc Natl Acad Sci U S A **96**(8): 4662-7.

Stocker, M. and P. Pedarzani (2000). "Differential distribution of three Ca^{2+}-activated K^+ channel subunits, SK1, SK2, and SK3, in the adult rat central nervous system." Mol Cell Neurosci **15**(5): 476-93.

Storm, J. F. (1989). "An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells." J Physiol **409**: 171-90.

Strack, S., M. A. Barban, B. E. Wadzinski and R. J. Colbran (1997). "Differential inactivation of postsynaptic density-associated and soluble Ca2+/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A." J Neurochem **68**(5): 2119-28.

Strassmaier, T., C. T. Bond, C. A. Sailer, H. G. Knaus, J. Maylie and J. P. Adelman (2005). "A novel isoform of SK2 assembles with other SK subunits in mouse brain." J Biol Chem.

Tacconi, S., R. Carletti, B. Bunnemann, C. Plumpton, E. Merlo Pich and G. C. Terstappen (2001). "Distribution of the messenger RNA for the small conductance calcium-activated potassium channel SK3 in the adult rat brain and correlation with immunoreactivity." Neuroscience **102**(1): 209-15.

Tang, Y. P., E. Shimizu, G. R. Dube, C. Rampon, G. A. Kerchner, M. Zhuo, G. Liu and J. Z. Tsien (1999). "Genetic enhancement of learning and memory in mice." Nature **401**(6748): 63-9.

Tsien, J. Z., P. T. Huerta and S. Tonegawa (1996). "The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory." Cell **87**(7): 1327-38.

Tzounopoulos, T. and R. W. Stackman (2003). "Enhancing synaptic plasticity and memory: a role for small conductance Ca2+-activated K+ channels." Neuroscientist **9**(6): 434-9.

Upchurch, M. and J. M. Wehner (1988). "Differences between inbred strains of mice in Morris water maze performance." Behav Genet **18**(1): 55-68.

van der Staay, F. J., R. J. Fanelli, A. Blokland and B. H. Schmidt (1999). "Behavioral effects of apamin, a selective inhibitor of the SK(Ca)- channel, in mice and rats." Neurosci Biobehav Rev **23**(8): 1087-110.

Vnek, N. and L. A. Rothblat (1996). "The hippocampus and long-term object memory in the rat." J Neurosci **16**(8): 2780-7.

Vogalis, F., J. F. Storm and B. Lancaster (2003). "SK channels and the varieties of slow after-hyperpolarizations in neurons." Eur J Neurosci **18**(12): 3155-66.

Wan, H., J. P. Aggleton and M. W. Brown (1999). "Different contributions of the hippocampus and perirhinal cortex to recognition memory." J Neurosci **19**(3): 1142-8.

Warburton, E. C., A. L. Baird, A. Morgan, J. L. Muir and J. P. Aggleton (2000). "Disconnecting hippocampal projections to the anterior thalamus produces deficits on tests of spatial memory in rats." Eur J Neurosci **12**(5): 1714-26.

Watanabe, S., D. A. Hoffman, M. Migliore and D. Johnston (2002). "Dendritic K+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons." Proc Natl Acad Sci U S A **99**(12): 8366-71.

Westphal, R. S., S. J. Tavalin, J. W. Lin, N. M. Alto, I. D. Fraser, L. K. Langeberg, M. Sheng and J. D. Scott (1999). "Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex." Science **285**(5424): 93-6.

Whishaw, I. Q. and H. Maaswinkel (1998). "Rats with fimbria-fornix lesions are impaired in path integration: a role for the hippocampus in "sense of direction"." J Neurosci **18**(8): 3050-8.

Wigstrom, H. and B. Gustafsson (1986). "Postsynaptic control of hippocampal long-term potentiation." J Physiol (Paris) **81**(4): 228-36.

Winder, D. G. and J. D. Sweatt (2001). "Roles of serine/threonine phosphatases in hippocampal synaptic plasticity." Nat Rev Neurosci **2**(7): 461-74.

Winters, B. D. and T. J. Bussey (2005). "Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory." J Neurosci **25**(1): 52-61.

Wood, E. R., P. A. Dudchenko and H. Eichenbaum (1999). "The global record of memory in hippocampal neuronal activity." Nature **397**(6720): 613-6.

Xia, X. M., B. Fakler, A. Rivard, G. Wayman, T. Johnson-Pais, J. E. Keen, T. Ishii, B. Hirschberg, C. T. Bond, S. Lutsenko, J. Maylie and J. P. Adelman (1998). "Mechanism of calcium gating in small-conductance calcium-activated potassium channels." Nature **395**(6701): 503-7.

Xiang, J. Z. and M. W. Brown (1999). "Differential neuronal responsiveness in primate perirhinal cortex and hippocampal formation during performance of a conditional visual discrimination task." Eur J Neurosci **11**(10): 3715-24.

Yang, S. N., Y. G. Tang and R. S. Zucker (1999). "Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation." J Neurophysiol **81**(2): 781-7.

Yeckel, M. F., A. Kapur and D. Johnston (1999). "Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism." Nat Neurosci **2**(7): 625-33.

Yuste, R., A. Majewska, S. S. Cash and W. Denk (1999). "Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis." J Neurosci **19**(6): 1976-87.

Zalutsky, R. A. and R. A. Nicoll (1990). "Comparison of two forms of long-term potentiation in single hippocampal neurons." Science **248**(4963): 1619-24.

Zelcer, I., H. Cohen, G. Richter-Levin, T. Lebiosn, T. Grossberger and E. Barkai (2005). "A Cellular Correlate of Learning-induced Metaplasticity in the Hippocampus." Cereb Cortex.

Zhang, W. and D. J. Linden (2003). "The other side of the engram: experience-driven changes in neuronal intrinsic excitability." Nat Rev Neurosci **4**(11): 885-900.

Zhu, X. O., B. J. McCabe, J. P. Aggleton and M. W. Brown (1996). "Mapping visual recognition memory through expression of the immediate early gene c-fos." Neuroreport **7**(11): 1871-5.

Zola, S. M. and L. R. Squire (2001). "Relationship between magnitude of damage to the hippocampus and impaired recognition memory in monkeys." Hippocampus **11**(2): 92-8.

Zola, S. M., L. R. Squire, E. Teng, L. Stefanacci, E. A. Buffalo and R. E. Clark (2000). "Impaired recognition memory in monkeys after damage limited to the hippocampal region." J Neurosci **20**(1): 451-63.

Zola-Morgan, S., L. R. Squire and D. G. Amaral (1986). "Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus." J Neurosci **6**(10): 2950-67.

Zola-Morgan, S., L. R. Squire and M. Mishkin (1982). "The neuroanatomy of amnesia: amygdala-hippocampus versus temporal stem." Science **218**(4579): 1337-9.

Zorumski, C. F., L. L. Thio, G. D. Clark and D. B. Clifford (1989). "Calcium influx through N-methyl-D-aspartate channels activates a potassium current in postnatal rat hippocampal neurons." Neurosci Lett **99**(3): 293-9.

Zucker, R. S. (1989). "Short-term synaptic plasticity." Annu Rev Neurosci **12**: 13-31.

APPENDIX A

The Journal of Neuroscience, December 1, 2002, 22(23):10163–10171

Small Conductance Ca^{2+}-Activated K^+ Channels Modulate Synaptic Plasticity and Memory Encoding

Robert W. Stackman,[2] Rebecca S. Hammond,[2] Eftihia Linardatos,[2] Aaron Gerlach,[1] James Maylie,[3] John P. Adelman,[1] and Thanos Tzounopoulos[1,2]

[1]Vollum Institute, Departments of [2]Behavioral Neuroscience and [3]Obstetrics and Gynecology, Oregon Health and Science University, Portland, Oregon 97239-3098

Activity-dependent changes in neuronal excitability and synaptic strength are thought to underlie memory encoding. In hippocampal CA1 neurons, small conductance Ca^{2+}-activated K^+ (SK) channels contribute to the afterhyperpolarization, affecting neuronal excitability. In the present study, we examined the effect of apamin-sensitive SK channels on the induction of hippocampal synaptic plasticity in response to a range of stimulation frequencies. In addition, the role of apamin-sensitive SK channels on hippocampal-dependent memory encoding and retention was also tested. The results show that blocking SK channels with apamin increased the excitability of hippocampal neurons and facilitated the induction of synaptic plasticity by shifting the modification threshold to lower frequencies. This facilitation was NMDA receptor (NMDAR) dependent and appeared to be postsynaptic. Mice treated with apamin demonstrated accelerated hippocampal-dependent spatial and nonspatial memory encoding. They required fewer trials to learn the location of a hidden platform in the Morris water maze and less time to encode object memory in an object-recognition task compared with saline-treated mice. Apamin did not influence long-term retention of spatial or nonspatial memory. These data support a role for SK channels in the modulation of hippocampal synaptic plasticity and hippocampal-dependent memory encoding.

Key words: synaptic plasticity; Ca^{2+}-activated K^+ channels; excitability; hippocampus; spatial memory; object memory

In hippocampal pyramidal neurons, action potentials are followed by an afterhyperpolarization (AHP) with three kinetic components. The predominant components, the medium AHP (mAHP) and slow AHP (sAHP), are attributable to the activation of small conductance Ca^{2+}-activated K^+ (SK) channels (Blatz and Magleby, 1986; Lancaster and Nicoll, 1987; Storm, 1990; Sah, 1996; Stocker et al., 1999). In addition to their different kinetics, the mAHP and the sAHP can be pharmacologically distinguished because apamin blocks the mAHP but not the sAHP (Kohler et al., 1996; Sah and Clements, 1999; Stocker et al., 1999). Apamin, a peptide derived from bee venom, is a highly selective blocker of SK channels, having no other known targets (Garcia et al., 1991). In CA1 neurons, synaptic activation may induce Ca^{2+} influx through NMDA receptors (NMDARs) (Alford et al., 1993; Kovalchuk et al., 2000), as well as through voltage-gated Ca^{2+} channels (Magee and Johnston, 1995). Synaptic activation of a Ca^{2+}-dependent K^+ current resembling the I_{sAHP} reduces postsynaptic excitability in response to high-frequency synaptic input (Lancaster et al., 2001).

Multiple forms of synaptic plasticity occur at the Schaffer collateral CA1 synapses, including long-term potentiation (LTP) and long-term depression (LTD) (Malenka and Nicoll, 1993). Essential for these processes is the influx of Ca^{2+} through NMDARs and the consequent rise in cytosolic Ca^{2+} (Lynch et al., 1983; Brocher et al., 1992; Malenka et al., 1992; Mulkey and Malenka, 1992). The magnitude of the rise in cytosolic Ca^{2+}, as determined by the degree and pattern of NMDAR activation, distinguishes whether a synapse undergoes LTP or LTD. Trains of afferent stimuli capable of inducing synaptic plasticity cause a summation of EPSPs that generate action potentials. The consequent increases in intracellular Ca^{2+} may activate SK channels; thus SK channels may represent a mechanism for modulating the induction of synaptic plasticity. Using a single stimulus frequency (100 Hz for 1 sec or 5 Hz for 3 min) (Behnisch and Reymann, 1998; Norris et al., 1998; Foster, 1999), the magnitude of LTP induced in the CA1 region was increased by extracellular application of apamin. The present experiments investigated whether SK channels modulate the threshold for synaptic plasticity as defined by the frequency–response function (Bear, 1995) and the mechanism through which such a modulation may occur. By using a wide range of stimulation frequencies, the results show that SK channel activity modulated the threshold for the induction of synaptic plasticity through a postsynaptic mechanism that required NMDAR activation.

SK channel blockade has been shown to (1) facilitate hippocampal-independent learning (Messier et al., 1991; Fournier et al., 2001) and (2) enhance spatial memory in hippocampal-lesioned mice but not in intact mice (Ikonen et al., 1998; Ikonen and Riekkinen, 1999). Differences in behavioral paradigm and the precise memory process addressed complicate the literature concerning the cognitive effects of apamin in rodents. Based on our electrophysiological findings, hippocampal-dependent tests were specifically modified to examine the effects of apamin on the

Received May 10, 2002; revised Sept. 24, 2002; accepted Sept. 24, 2002.

This work was supported by a grant from the Medical Research Foundation of Oregon (T.T.) and by grants from the National Institutes of Health (J.M., J.P.A.). We thank Drs. Craig Jahr, Laurence Trussell, and members of their laboratories for helpful discussions. We also thank Sophie Davis (Oregon Health and Science University/Portland State University Saturday Academy 2001 summer high school science apprenticeship program) for help with behavioral testing and Laura Tull, Dr. Anastassios Tzingounis, and Dr. Jacques Wadiche for helpful comments on this manuscript.

Correspondence should be addressed to Dr. Thanos Tzounopoulos, Auditory Neuroscience, L-335A, Oregon Hearing Research Center, Oregon Health and Science University, 3181 Southwest Sam Jackson Park Road, Portland, OR 97239-3098. E-mail: tzounopo@ohsu.edu.

 0270-6474/02/2210163-09$15.00/0

initial stages of memory encoding. The data demonstrate that apamin facilitated spatial and nonspatial memory encoding in C57BL/6 mice.

MATERIALS AND METHODS

Electrophysiology

Hippocampal slices were prepared from 3- to 6-week-old male C57BL/6NHsd mice (Harlan Sprague Dawley, Indianapolis, IN). Animals were anesthetized with halothane and decapitated. The cerebral hemispheres were quickly removed and placed in a partially frozen solution of artificial CSF (ACSF) (in mM): 119 NaCl, 2.5 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1 $NaHPO_4$, 26.2 $NaHCO_3$, and 10 glucose and equilibrated with 95% O_2 and 5% CO_2. Hippocampi were removed, placed on an agar block, and transferred to a slicing chamber containing a similarly partially frozen solution. Transverse hippocampal slices (300–500 μm thick) were cut with a Vibratome tissue slicer, transferred into a humidified holding chamber, and allowed to recover for ≥1 hr before recordings were performed. The following drugs were used: apamin (Calbiochem, La Jolla, CA) and D-2 amino-5-phosphonovaleric acid (D-APV) (Tocris Cookson, Ellisville, MO). Extracellular field potentials were recorded in the stratum radiatum using electrodes (3–6 MΩ) filled with 3 M NaCl. For whole-cell recordings, CA1 pyramidal neurons were visualized with a water-immersion objective (40×; Zeiss, Thornwood, NY) using a microscope equipped with infrared/differential interference contrast optics (Zeiss Axioskop 2FS) and a CCD camera (Sony, Tokyo, Japan). Whole-cell recording pipettes were fabricated from TW150F-4 thin-wall borosilicate glass (World Precision Instruments, Sarasota, FL) and had resistances of 1.5–3 MΩ. Pipettes were filled with an intracellular solution containing (in mM): 140 $KMeSO_4$, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 2 Mg-ATP, 0.4 Na_2-GTP, and 20 μM EGTA, pH 7.3, 290 mOsm. Slices were continuously perfused with ACSF. Whole-cell, patch-clamp currents were recorded with 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). CA1 neurons were voltage clamped at −55 mV, and I_{AHP} tail currents were evoked by a depolarizing voltage command to +20 mV for 200 msec followed by a return to −55 mV. Experiments on control slices were interleaved with those on experimental slices. Data were collected and analyzed online (10 kHz sampling rate) using IGOR (WaveTech, Lake Oswego, OR) and a program kindly donated by Dr. Greg Hjelmstad (University of California San Francisco, San Francisco, CA). The maximal initial slope of the field EPSP was measured to monitor the strength of synaptic transmission, minimizing contamination by voltage-dependent events. Summary graphs were obtained by normalizing each experiment according to the average value of all points on the 10 min baseline, aligning the points with respect to the start of the (LTP and LTD) induction protocol, dividing each experiment into 1 min bins, and averaging these across experiments. The amount of potentiation or depression of the synaptic response was measured 40–50 min after conditioning. Data are expressed as mean ± SEM, as a percentage of the baseline. Student's t test and two-factor ANOVA were used to determine significance between groups of data; $p < 0.05$ was considered significant. Experiments were included in the data analysis only when LTP could be generated at the end of the experimental manipulation, ensuring that the occurrence of short-term potentiation or LTD was attributable to the experimental manipulation.

Morris water maze

To assess hippocampal-dependent spatial learning and memory, naive male C57BL/6NHsd mice (4–6 weeks of age) were trained in a Morris water maze (Silva et al., 1998; Cho et al., 1999). Before the start of behavioral testing, mice were habituated over a 3 d period to daily handling and intraperitoneal injection. Over the following 2 d, all mice received nonspatial habituation trials (one trial per day). During these trials, a clear Plexiglas platform (13 cm diameter) was placed in the center of a white polyethylene pool (60 cm high, 109 cm diameter), and floor-to-ceiling curtains were drawn around the pool to block the animals' use of extra-maze cues. The platform was 1 cm below the surface of the water, and the water was rendered opaque by the addition of nontoxic white Tempra paint. Each mouse was placed on the platform for 60 sec and then released into the pool at four locations adjacent to the platform and allowed to swim and climb onto the platform.

Spatial training. After nonspatial habituation, mice were trained on the spatial (hippocampal-dependent) version of the water maze task. Training comprised 24 trials (four trials per day) during which the platform remained submerged 1 cm below the water surface in a fixed position in the center of one quadrant of the pool. During a given trial, the mouse was introduced into the pool at one of four possible start points (north, south, west, and east) and allowed 60 sec to swim to the platform. The order of start points varied in a pseudorandom manner for each mouse every day. After remaining on the platform for 30 sec, the mouse was placed into a holding cage for a 45 sec intertrial interval. Throughout water maze testing, the water temperature was maintained at 22–23°C. Each mouse received intraperitoneal apamin (0.4 mg/kg, 10 ml/kg; Calbiochem) or 0.9% saline (10 ml/kg) 30 min before the first training trial of each day. This dose of apamin was defined in pilot studies conducted to determine a dose that was behaviorally effective but that induced no motor or convulsive effects.

Spatial memory testing. After the fourth, 12th, and 20th training trials, a probe test was conducted in which each mouse received a 30 sec free swim in the pool with the platform removed. Twenty-four hours after the final training trial (24th trial), each mouse received a 60 sec probe test of long-term retention. The behavior of the mice during training and probe tests was recorded with a computerized video tracking system (EthoVision 2.2; Noldus, Leesburg, VA) and analyzed to determine the amount of time spent in each of the four quadrants of the water maze.

Object recognition

To assess the effects of apamin on nonspatial hippocampal-dependent memory, naive male C57BL/6NHsd mice (4–6 weeks of age) were tested in an object-recognition memory task (Vnek and Rothblat, 1996; Clark et al., 2000). Before object recognition testing, all mice were habituated to intraperitoneal injection and to the open-field arena (38 × 38 × 64 cm high) for 5 min each day for 3 d. During a subsequent sample session, two identical novel objects [Duplo or Lego blocks (Lego Company, Billund, Denmark), toys, etc.] were placed into opposite corners (southwest and northeast) of the open-field arena, and the mouse was allowed to explore the objects. Pilot studies revealed that C57BL/6 mice averaged 38 sec of exploration of either sample object during a 5 min sample session. For the present study, the object recognition task was modified to explicitly examine the influence of apamin on object memory encoding. To manipulate encoding, mice were allowed to explore the sample objects until either 19 sec (minimal training) or 38 sec (extensive training) of object exploration had been accumulated. Twenty-four hours after the sample session, a test session was conducted during which each mouse was placed back into the arena containing one of the familiar objects and a novel object for 5 min. The spatial position of the novel object was counterbalanced so that one-half of the mice experienced the novel object in the southwest corner of the open field, whereas the other half of the mice experienced the novel object in the northeast corner. After each session, all objects were cleaned with 10% ethanol to reduce the possibility that mice were imparting some odor cue to the objects that would influence object exploratory behavior during a subsequent test session. Pilot studies were conducted to select objects that elicited equivalent degrees of exploration in mice. This is necessary to verify that naive mice exhibited no inherent preference for one object over the other.

The behavior of each mouse was recorded using the EthoVision system and scored to determine the amount of time spent exploring each of the objects during each session. Object exploration was defined as any time that the mouse's head was oriented toward the object, was within 2–3 cm of the object, and its vibrissas were moving. Object recognition memory was quantified by measuring the difference in exploration times between the novel and familiar object. A novel object preference index, a ratio of the amount of time spent exploring the novel object over the total time spent exploring both objects, was used to measure recognition memory. A novel object preference ratio of >0.5 indicates that the mouse spent more time exploring the novel object than the familiar one.

RESULTS

Apamin blocks SK channels underlying the mAHP and increases excitability

CA1 neurons express an apamin-sensitive I_{mAHP} (Fig. 1*A*) thought to be mediated by apamin-sensitive SK channels (Kohler et al., 1996; Stocker et al., 1999). Action potentials recorded in response to current injections showed that apamin (100 nM) increased the number of action potentials discharged in CA1

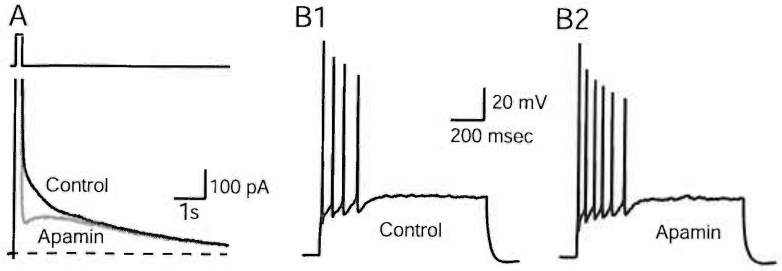


Figure 1. Blockade of the apamin-sensitive afterhyperpolarization (mAHP) increases excitability. *A*, I_{AHPs} were evoked in the whole-cell configuration by a 200 msec depolarizing pulse to +20 mV followed by a return to the −55 mV holding potential. I_{AHPs} were obtained in the presence and absence of apamin (100 nM). After application of apamin, the medium-duration component (I_{mAHP}) of the tail current was selectively inhibited. *Dashed line* indicates zero current. *B*, Apamin increased the number of action potentials. *B1*, Response of a pyramidal neuron to a 1 sec depolarizing current pulse. *B2*, Response of the same neuron to the same depolarizing current pulse in the presence of apamin (control cells fired an average ± SEM of 4.7 ± 1.2 action potentials/depolarizing pulse, which increased to 6.7 ± 1.7 with apamin; $n = 5$; $p = 0.04$; paired Student's t test).

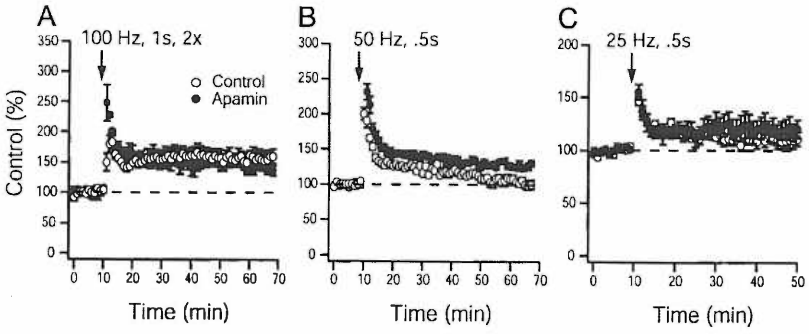


Figure 2. Apamin block of SK channel activity enhances plasticity induced by high-frequency stimulation. *A*, A 100 Hz, 1 sec tetanus in control and apamin (100 nM)-treated slices (164 ± 7%, $n = 9$ slices per 6 animals for controls; 165 ± 6%, $n = 10$ slices per 6 animals for apamin; $p > 0.05$; unpaired Student's t test). *B*, A 50 Hz, 0.5 sec stimulation protocol in control and apamin (100 nM)-treated slices (106 ± 4%, $n = 12$ slices per 8 animals for control slices, 125 ± 3%, $n = 13$ slices per 8 animals for apamin-treated slices; $p < 0.05$; unpaired Student's t test). *C*, A 25 Hz, 0.5 sec stimulation protocol in control and apamin (100 nM)-treated slices (109 ± 9%, $n = 8$ slices per 6 animals for controls; 120 ± 6%, $n = 8$ slices per 6 animals for apamin; $p > 0.05$; unpaired Student's t test). Control and apamin-treated slices were interleaved. Synaptic strength was measured as the initial slope of the recorded field EPSP. *Dashed line* indicates baseline response in *A–C*.

neurons (Fig. 1*B*). Control cells fired an average ± SEM of 4.7 ± 1.2 action potentials per depolarizing pulse (Fig. 1*B1*), which was increased to 6.7 ± 1.7 in the presence of apamin (Fig. 1*B2*) ($n = 5$; $p = 0.04$; paired Student's t test). This result indicates that blockade of apamin-sensitive SK channels increases excitability. Such changes in excitability may influence the threshold for the induction of synaptic plasticity.

Blocking SK channels facilitates the induction of synaptic plasticity

To investigate the role of SK channels on the induction of synaptic plasticity at CA1 synapses, stimulation protocols that evoke LTP or LTD were delivered to mouse hippocampal brain slices in the presence or absence of apamin. Figure 2*A* shows the effect of apamin (100 nM) application on the ability of high-frequency stimulation (100 Hz applied twice for 1 sec, separated by 10 sec) to generate LTP. Equal extents of LTP were observed in control (164 ± 7%; $n = 9$ slices per 6 animals) and apamin-treated (165 ± 6%; $n = 10$ slices per 6 animals) slices, showing that apamin does not alter the ability of high-frequency stimulation to induce robust LTP ($p > 0.05$; unpaired Student's t test). After a 50 Hz, 0.5 sec stimulus, significantly more LTP was induced in the presence of apamin (125 ± 3%, $n = 13$ slices per 8 animals for apamin-treated slices; 106 ± 4%, $n = 12$ slices per 8 animals for control slices; $p < 0.05$; unpaired Student's t test) (Fig. 2*B*). Using a 25 Hz, 0.5 sec stimulus, LTP was not different in control and apamin-treated slices (120 ± 6%, $n = 8$ slices per 6 animals for apamin-treated slices; 109 ± 9%, $n = 8$ slices per 6 animals for control slices; $p > 0.05$; unpaired Student's t test) (Fig. 2*C*).

To determine whether apamin affects the threshold for induction of synaptic plasticity, its effects on lower stimulation frequencies were examined. A 10 Hz stimulation for 900 pulses resulted in LTD in control slices (77 ± 6%; $n = 10$ slices per 6 animals), whereas apamin-treated slices did not show changes in synaptic strength (101 ± 7%; $n = 9$ slices per 5 animals; $p < 0.05$; unpaired Student's t test) (Fig. 3*A*). In addition, 5-Hz stimulation for 900 pulses resulted in LTD in apamin-treated slices (85 ± 5%; $n = 9$ slices per 5 animals) but did not affect long-lasting changes in synaptic strength in control slices (103 ± 4%; $n = 10$ slices per 6 animals; $p < 0.05$; unpaired Student's t test) (Fig. 3*B*). These results suggest that apamin alters the frequency–response relationship (Bear, 1995) for the induction of synaptic plasticity. The modification threshold is the level of postsynaptic response at which the sign of the synaptic modification reverses from LTD to LTP (Bienenstock et al., 1982). The smooth transition from LTD to LTP may be demonstrated by systematically varying the frequency of conditioning stimulation for a given number of pulses. The frequency–response relationships for control and apamin-treated slices are presented in Figure 3*C* and demonstrate that blockade of SK channels with apamin shifts the frequency–response function to the lower frequencies, facilitating the induction of synaptic plasticity.

Blocking SK channels does not affect neurotransmitter release

To investigate whether the apamin-induced shift in the frequency–response function at CA1 synapses involves presynaptic or postsynaptic changes, the effects of apamin on paired-pulse facilitation, post-tetanic potentiation, and short-term depression were investigated.

Paired-pulse facilitation, an increased second response to two stimuli applied in rapid succession, is thought to reflect an increase in the probability of neurotransmitter release (Katz and Miledi, 1968). Paired-pulse facilitation was tested at interstimulus

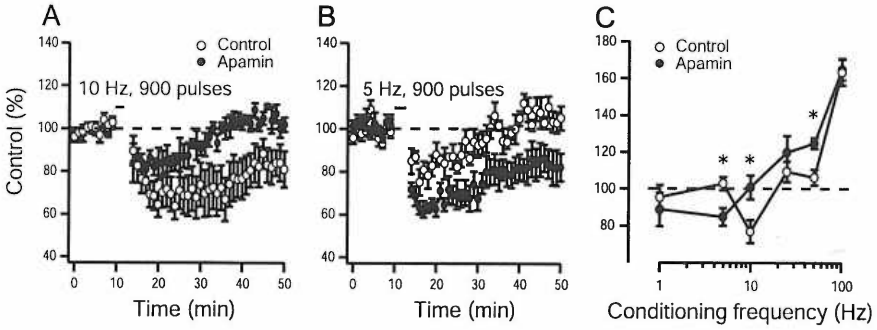


Figure 3. Apamin block of SK channel activity shifts the synaptic modification threshold to lower frequencies. Induction of synaptic plasticity by 10 Hz, 900 pulse stimulation in control slices (77 ± 6%; n = 10 slices per 6 animals) and apamin (100 nM)-treated slices (101 ± 7%; n = 9 slices per 5 animals; $p < 0.05$; unpaired Student's t test) (*A*) and 5 Hz, 900 pulse stimulation protocol in control slices (103 ± 4%; n = 8 slices per 5 animals) and apamin (100 nM)-treated slices (85 ± 5%; n = 9 slices per 5 animals; $p < 0.05$; unpaired Student's t test) (*B*). *Dashed line* indicates baseline response. *C*, Frequency–response relationship for the induction of LTP and LTD in controls and experiments from slices in which apamin (100 nM) was applied. The mean effect of 900 pulses of conditioning stimulation delivered at various frequencies to the Shaffer collaterals on the synaptic response measured 40–50 min after conditioning is shown. *$p < 0.05$ versus respective control data point; Student's t test. *Dashed line* indicates the transition between LTD and LTP.

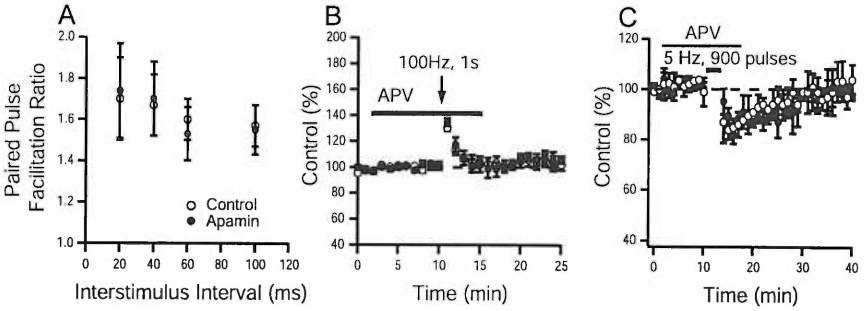


Figure 4. SK channels do not have presynaptic effects in CA1. *A*, Paired-pulse facilitation (*PPF*), measured as the ratio of the second response to the first, was plotted as a function of interstimulus interval for controls and in the presence of apamin ($n > 8$ for all interstimulus intervals). No significant differences were detected ($p > 0.05$; paired Student's t test). *B*, Time course of post-tetanic potentiation elicited by 100 Hz, 1 sec tetanus in control and apamin-treated slices. Post-tetanic potentiation (peak enhancement in controls, 132 ± 6% of baseline, n = 9 slices per 4 animals; peak enhancement in apamin, 134 ± 5% of baseline, n = 9 slices per 4 animals) was not different between groups ($p > 0.05$; unpaired Student's t test). *C*, Time course of short-term depression elicited by 5 Hz, 900 pulse stimulation in control and apamin-treated slices (80 ± 7% of baseline, n = 6 slices per 3 animals; peak depression in apamin, 82 ± 5% of baseline, n = 6 slices per 3 animals). No significant differences were detected between groups ($p > 0.05$; paired Student's t test). Synaptic strength was measured as the initial slope of the recorded field EPSP. *Solid line* in *B* and *C* indicates the duration of D-APV application.

intervals ranging from 20 to 100 msec and was not significantly altered by application of apamin ($p > 0.05$; paired Student's t test; $n > 8$ for all interstimulus intervals) (Fig. 4*A*), suggesting that apamin does not alter neurotransmitter release.

Post-tetanic potentiation, a slow decay of the postsynaptic responses after repetitive stimulation has been terminated, presumably reflects the slow decay of elevated presynaptic Ca^{2+} levels induced by the tetanic stimulus (Zucker, 1989). The effects of apamin on post-tetanic potentiation were examined using a 100 Hz, 1 sec tetanus delivered in the presence of D-APV (100 μM), an NMDA receptor antagonist. The time course of post-tetanic potentiation was not different between control and apamin-treated slices, nor were differences detected in the peak enhancement achieved in the presence or absence of apamin (control, 132 ± 6%, n = 9 slices per 4 animals; apamin treated, 134 ± 5%, n = 9 slices per 5 animals; $p > 0.05$; unpaired Student's t test) (Fig. 4*B*).

Short-term depression was also examined using a 5 Hz, 900 stimuli tetanus delivered in the presence of D-APV (100 mM). This same protocol had revealed differences between control and apamin-treated slices when performed in the absence of D-APV (Fig. 3*B*). In the presence of D-APV (Fig. 4*C*), the magnitude and time course of depression were not different between control slices (peak depression, 80 ± 7%, n = 9 slices per 4 animals) and apamin-treated slices (peak depression, 82 ± 5%, n = 9 slices per 5 animals; $p > 0.05$; unpaired Student's t test). The lack of effect of apamin on paired-pulse facilitation, post-tetanic potentiation, and short-term depression suggests that apamin does not affect presynaptic events but rather alters NMDAR-dependent postsynaptic events to shift the threshold for the induction of synaptic plasticity in CA1 synapses.

Blocking SK channels accelerates hippocampal-dependent spatial memory encoding

The results presented above indicate that apamin facilitates the induction of synaptic plasticity in the CA1 region of the hippocampus. It was hypothesized that apamin might also alter hippocampal-dependent memory assessed in the Morris water maze, a task considered to require the activation of NMDARs and synaptic plasticity in the hippocampus (Morris et al., 1982; Tsien et al., 1996). Considering the finding that apamin shifted the threshold for the induction of synaptic plasticity (Fig. 3*C*), it was predicted that apamin would exert its greatest influence during the initial stages of spatial memory encoding. Specifically, the effects of systemic apamin were examined using a version of the Morris water-maze task modified to explicitly assess the encoding of spatial memory. The rationale being that if synaptic plasticity is more easily induced in the presence of apamin, fewer trials may be required to encode spatial memory in apamin-treated mice. Naive mice received apamin (0.4 mg/kg, i.p.; n = 10) or 0.9% saline (n = 9) 30 min before daily training for 6 d (four trials per day) in the water maze task. The platform location remained fixed throughout all training trials. Immediately after the fourth, 12th, and 20th training trials, each mouse received a 30 sec probe test. These interpolated probe tests assess the development of a spatial bias for the training quadrant of the pool at an

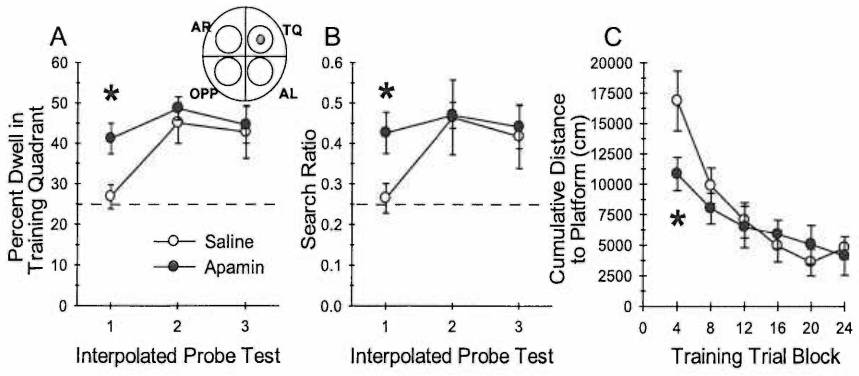


Figure 5. Apamin block of SK channels facilitates the encoding of spatial memory. *A*, A modified Morris water maze task was used to examine the effects of apamin on encoding of spatial memory. Mice were trained for four trials per day for 6 d, and 30 sec probe tests were presented immediately after the fourth, 12th, and 20th trial. Mean ± SEM percentage of time spent dwelling (*Percent Dwell*) in the training quadrant during the interpolated probe tests revealed that mice treated with 0.4 mg/kg apamin (n = 10) spent significantly more time in the training quadrant during the first probe test than saline-treated (n = 9) control mice (*p < 0.009 vs saline-treated mice on probe test 1; planned comparison Student's t test). The *dashed line* at 25% represents chance performance. *AL*, Adjacent left; *AR*, adjacent right; *OPP*, opposite; *TQ*, training quadrant. *B*, Mean ± SEM search ratio reflects the accuracy with which mice search in the correct location within the training quadrant of the pool. Search ratio is computed as the number of times the animal crosses into the zone (see *circular regions* of *inset diagram*) encompassing the platform (*shaded zone*) divided by the total number of crossings into all four zones. The *dashed line* at 0.25 represents chance performance during the probe tests or the lack of spatial bias for any particular pool location. Apamin-treated mice exhibited a significantly higher search ratio than saline-treated mice during the first probe test (*p < 0.02 vs saline-treated mice on probe test 1; planned comparison Student's t test). Measures of the percentage of time spent dwelling in the training quadrant or search ratio from the second or third probe tests were equivalent between the two groups, indicating that there were no group differences in platform search behavior after more training. *C*, Mean ± SEM cumulative distance to platform measures of saline- and apamin-treated mice plotted in blocks of four training trials. This measure indicates the proximity of the mice to the platform during each training trial. Consistent with the data from probe test 1, apamin-treated mice swam in closer proximity to the platform during the first four trial block of training than saline-treated mice (*p < 0.04; *post hoc* Tukey multiple comparisons test).

early (probe 1), intermediate (probe 2), and late (probe 3) stage of spatial memory encoding. Apamin treatment accelerated the development of a spatial bias for the training quadrant during the first interpolated probe test (probe 1), as shown in Figure 5*A*. Planned comparisons analyses revealed that apamin-treated mice spent significantly more time in the training quadrant than saline-treated mice (mean ± SEM; apamin, 41.1 ± 3.8%; saline, 26.8 ± 2.9%; $t_{(17)} = -2.94$; $p = 0.009$). In addition, apamin-treated mice exhibited more accurate search behavior as indicated by search ratio (Fig. 5*B*), computed as the number of crossings into a circular zone encompassing the platform divided by the total number of crossings into all four zones (Fig. 5*B*, *inset diagram*) (apamin, 0.43 ± 0.05; saline, 0.26 ± 0.04; $t_{(17)} = -2.52$; $p = 0.02$). Saline-treated mice required 12 training trials to develop this degree of preference (probe 2). Thus, after minimal training (just four trials), apamin-treated mice exhibited significant spatial memory of the training quadrant, whereas control mice exhibited a chance level of performance. There were no additional differences in performance on probes 2 and 3 between apamin- and saline-treated mice, indicating that after 12 training trials, the saline-treated mice had acquired the memory for platform location and were performing as accurately as apamin-treated mice. Spatial memory encoded by apamin-treated mice was stable throughout the training session, because there was no difference in training-quadrant preference across the three interpolated probe tests. Two-factor, repeated-measures (treatment × four trial block) ANOVA on cumulative distance to platform measures revealed a significant treatment × four trial block interaction ($F_{(4,68)} = 2.53$; $p < 0.05$) and a significant effect of four trial block ($F_{(4,68)} = 15.75$; $p < 0.001$). Tukey multiple comparisons tests revealed a significant difference between apamin- and saline-treated mice in cumulative distance to platform on the first four trial block (Fig. 5*C*). The cumulative distance to platform is a score of the proximity of the mouse to the platform during training and is a more sensitive measure of spatial behavior than escape latency (Gallagher et al., 1993). The difference in cumulative distance measures between apamin- and saline-treated mice reflects more accurate platform search behavior by the apamin-treated mice, a finding that is consistent with the observed differences in spatial search behavior during probe 1. An identical analysis of escape latency data found a significant effect of four trial block ($F_{(4,68)} = 8.50$; $p < 0.001$) but no treatment × four trial block interaction ($F_{(4,68)} = 0.44$; $p > 0.5$) and no significant effect of treatment ($F_{(4,68)} = 0.54$; $p > 0.5$). Apamin treatment did not cause any overt influence on swimming, and swim speeds were not different between groups ($F_{(1,17)} = 0.29$; $p > 0.5$). Analyses restricted to the data from the first four training trials also indicated no significant differences in escape latencies ($t_{(17)} = 0.33$; $p > 0.05$) or swim speed ($t_{(17)} = 0.04$; $p > 0.05$). Collectively, these results suggest that apamin-mediated blockade of SK channels facilitated the encoding of hippocampal-dependent spatial memory.

Data from the probe test given 24 hr after the final training trial were examined to test whether memory encoded under SK channel blockade would be differentially retained. Both groups of mice exhibited a spatial bias for searching in the training quadrant during the 24 hr retention probe test. There were no differences between saline- and apamin-treated mice during the final probe trial with regard to the percentage of time spent dwelling in the training quadrant (mean ± SEM; saline, 41.6 ± 4.6; apamin, 38.1 ± 4.3; $t_{(17)} = 0.57$; $p > 0.6$) or with regard to the search ratio (saline, 0.43 ± 0.05; apamin 0.38 ± 0.05; $t_{(17)} = 0.64$; $p > 0.5$). Together with the data of Figure 5*A–C*, it appears that apamin-treated mice encoded the spatial memory of the platform location with less training than the saline-treated mice. However, once encoded, there was no difference in retention of the spatial memory between apamin- and saline-treated mice.

Blocking SK channels accelerates hippocampal-dependent nonspatial memory encoding.

To further examine the role of SK channels in hippocampal memory, the effects of apamin on a nonspatial object-recognition task were examined. This task assesses the encoding and retention of memory for an object and is sensitive to lesions of the hippocampus (Vnek and Rothblat, 1996; Clark et al., 2000) and to manipulation of hippocampal NMDARs (Tang et al., 1999).

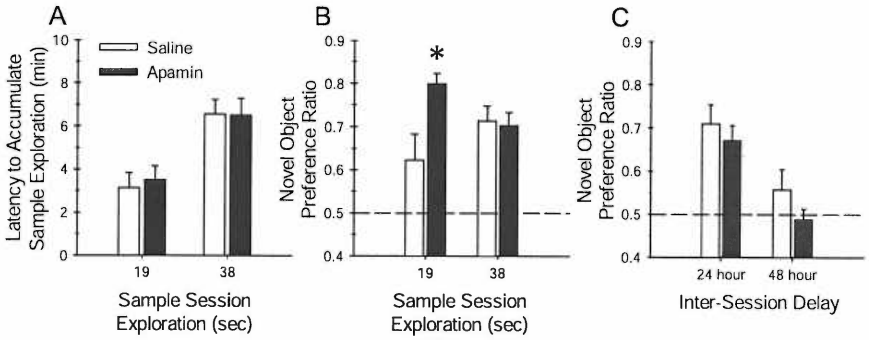


Figure 6. Apamin block of SK channel activity facilitates the encoding of nonspatial object memory but does not influence the retention of object memory. Object-recognition memory was quantified by computing the novel object preference ratio, the amount of time spent exploring the novel object during the test session divided by the total time spent exploring both the familiar and novel object. *A*, The object recognition task was modified to test the influence of apamin on object memory encoding. As described in Materials and Methods, during the sample session, saline- and apamin-treated mice were restricted to either 19 sec (minimal training) or 38 sec (extensive training) of sample object exploration. The amount of time required to accumulate either 19 or 38 sec of sample object exploration did not differ between apamin- and saline-treated mice (p values >0.05; unpaired Student's t test). *B*, Restricting the amount of object exploration during the sample session to 19 sec weakens the degree of preference exhibited by the mouse during a test session 24 hr later. This is illustrated by the lower novel object preference ratio of the saline-treated mice ($n = 9$) limited to 19 sec of sample object exploration. However, apamin (0.4 mg/kg)-treated mice ($n = 10$) that were limited to only 19 sec of sample object exploration exhibited a significantly greater novel object preference during the 24 hr test session ($*p < 0.04$ vs saline-treated mice permitted 19 sec of object exploration; planned comparison Student's t test). When apamin (0.4 mg/kg)-treated ($n = 10$) and saline-treated ($n = 9$) mice were permitted 38 sec of sample object exploration, there was no difference in novel object preference ratio during the 24 hr test session. Each *dashed line* at 0.5 represents chance performance or a lack of discrimination between the novel and familiar object. *C*, Object memory retention decays over a similar time course in apamin- and saline-treated mice. Both apamin- and saline-treated mice exhibited similar strong preference for the novel object during a 24 hr retention test; mean ± SEM novel object preference ratios were not significantly different. Four days later, the same mice received a second sample session with two new objects. When tested for retention 48 hr later, both apamin- and saline-treated mice failed to show a strong preference for the novel object over the familiar object. These data indicate that apamin does not affect the retention of object memory.

Given that apamin-treated mice developed a significant spatial memory for the platform location after minimal water-maze training, it was hypothesized that apamin would influence the encoding of object memory in a similar manner. Pilot studies indicated that during a 5 min sample session, C57BL/6J mice typically spend an average of 38 sec exploring each sample object. To examine the influence of apamin on object memory encoding, the amount of sample object exploration was manipulated. Mice were allowed to explore the sample objects until they had accumulated object exploration times of either 19 sec (minimal training) or 38 sec (extensive training). The results obtained for spatial memory encoding led to our prediction that apamin would facilitate object memory retention in mice limited to 19 sec of sample object exploration compared with respective saline-treated mice.

Naive C57BL/6NHsd mice received apamin (0.4 mg/kg, i.p.) or 0.9% saline 30 min before the sample session. Each mouse was placed into the arena containing two identical novel objects. Depending on group assignment, the mouse was removed from the arena after exploring either sample object for 19 sec (minimal training) or 38 sec (extensive training). During the sample session, there was no significant difference between saline- and apamin-treated mice with regard to the time required to reach the respective 19 or 38 sec sample object exploration limit ($t_{(17)} = -1.31$ and 0.04, respectively; p values of >0.05) (Fig. 6*A*), indicating that all mice exhibited the same curiosity and motivation. Object memory retention was assessed during a test session 24 hr later, in which each mouse was allowed to explore the arena containing one of the familiar objects from the sample session and a novel object. Saline-treated mice limited to 19 sec of sample object exploration exhibited a weaker preference for the novel object during the test session compared with mice permitted 38 sec of sample object exploration (Fig. 6*B*). Planned comparisons analysis revealed that apamin-treated mice limited to 19 sec of sample object exploration exhibited a stronger preference for the novel object compared with the respective saline-treated mice ($t_{(17)} = -2.17$; $p = 0.04$) (Fig. 6*B*). These data suggest that apamin is capable of facilitating object memory encoding. There was no difference in novel object preference ratio between saline- and apamin-treated mice permitted 38 sec of sample object exploration ($t_{(17)} = -0.11$; $p > 0.05$), indicating that both groups exhibited equivalent object memory retention. In accordance with our findings of apamin-treated mice in the Morris water-maze task, these findings suggest that apamin block of SK channels facilitates the encoding of nonspatial memory, perhaps by reducing the threshold for memory formation.

The retention of object memory decays faster in hippocampal-lesioned rodents (Vnek and Rothblat, 1996; Clark et al., 2000) and is sensitive to genetic manipulation of the hippocampal NMDAR (Tang et al., 1999). Hippocampal-lesioned rats fail to retain object memory over a 24 hr delay (Clark et al., 2000) but are able to retain object memory over a 5 min delay (Mumby et al., 2002). The influence of systemic apamin on the rate of decay of object memory retention was examined in a second cohort of C57BL/6NHsd mice. During a sample session, apamin- (0.4 mg/kg, i.p.) and saline-treated mice were exposed to two identical sample objects for 5 min. Both groups exhibited a similar preference for the novel object during the 24 hr test session, as shown in Figure 6*C*, indicating that apamin did not influence memory retention at 24 hr, consistent with the 38 sec data of Figure 6*B*. Four days later, the same groups were exposed to a second set of sample objects and then tested for retention 48 hr later. As depicted in Figure 6*C*, neither group exhibited a significant preference for the novel object at the 48 hr test session, suggesting that object memory decayed over the same rate between the two groups. These data indicate that apamin did not influence object memory retention.

DISCUSSION

The present study demonstrates that blockade of synaptically activated SK channels increases excitability and decreases the threshold for the induction of hippocampal synaptic plasticity via a postsynaptic mechanism that requires the activation of NMDARs. The reduced threshold for induction of synaptic plasticity is associated with facilitated memory encoding. This enhancement is correlated with changes in the induction of synaptic plasticity but is not necessarily attributable to these changes,

because there is no way to rule out the effects of apamin on other brain structures that can influence hippocampal function.

Neural circuits derive flexibility from activity-driven bidirectional modification of synaptic strength (Sejnowski, 1977; Bienenstock et al., 1982). An important characteristic of this process is the threshold for synaptic modification (Bear, 1995), which is defined by the frequency–response function for the induction of synaptic plasticity. As postsynaptic activity increases, the threshold for LTD is reached first, and an additional increase leads to a transition from LTD to LTP. This transition represents the synaptic modification threshold (Bear, 1995). A prominent model for the regulation of the synaptic modification threshold proposes that the direction of altered synaptic efficacy, potentiation, or depression is determined by the level of postsynaptic Ca^{2+} during neural activity (Lisman, 1989; Artola and Singer, 1993; Malenka and Nicoll, 1993). The rise in Ca^{2+} within the dendritic spine is the critical trigger for synaptic plasticity. Stronger depolarization allows more Ca^{2+} to enter and leads to synaptic potentiation (Lisman, 1989; Artola and Singer, 1993; Bliss and Collingridge, 1993; Cummings et al., 1996; Malenka and Nicoll, 1999), whereas weaker depolarization leads to less Ca^{2+} influx and synaptic depression (Mulkey and Malenka, 1992; Dudek and Bear, 1993). Therefore, any manipulation that influences the magnitude or dynamics of Ca^{2+} increase within dendritic spines may profoundly influence the form of the resulting synaptic plasticity.

Synaptic activation of the channels underlying the I_{sAHP} (Lancaster et al., 2001; Martin et al., 2001) regulates synaptic efficacy and may influence the threshold for synaptic plasticity, as hypothesized by previous studies (Sah and Bekkers, 1996). Our results showed that application of apamin caused a shift of the synaptic modification threshold to lower frequencies, an effect that is consistent with facilitated induction of synaptic plasticity. Apamin-sensitive SK channels underlie the mAHP in CA1 neurons, which peaks ~200 msec after the action potential (Sah and Clements, 1999; Stocker et al., 1999), a time course that may enable the mAHP to influence neuronal discharge activity, and the integration of synaptic events as the rate of afferent stimulation increases toward the threshold for synaptic plasticity (5–20 Hz). These are precisely the stimulation frequencies around which apamin exerted its significant effects on the induction of synaptic plasticity.

Our results suggest that SK channel activity modulates the induction of synaptic plasticity that requires postsynaptic depolarization and NMDAR activation. Postsynaptic depolarization induced by repetitive synaptic stimulation raises intracellular Ca^{2+} levels through voltage-gated Ca^{2+} channels or NMDARs, permitting the activation of SK channels. By hyperpolarizing the postsynaptic membrane, SK channels decrease excitability and modulate the activation of NMDARs, which involves voltage-dependent removal of the Mg^{2+} block (Mayer et al., 1987). By affecting the degree of NMDAR activation and the subsequent Ca^{2+} entry, SK channels may modulate the induction of synaptic plasticity. Our experiments suggest that SK channels are dendritically localized. Although direct evidence for the distribution of SK channels in the dendrites is currently unavailable, it has been suggested that apamin-sensitive SK channels are located predominantly in proximal and distal dendrites of motor neurons (Cangiano et al., 2002). In addition, Ca^{2+}-activated K^+ channels have been reported in the dendrites of mammalian neurons (Andreasen and Lambert, 1995; Sah and Bekkers, 1996; Schwindt and Crill, 1997).

Synaptic plasticity is believed to represent, at least in part, the cellular mechanisms responsible for learning and memory. It is generally accepted that some form of an increase in synaptic efficacy in the hippocampus is necessary for encoding spatial memory in the water maze task (Moser et al., 1998). Whether such memory formation in the hippocampus is dependent on LTP or LTD has been difficult to establish (Holscher, 1997; Jeffery, 1997; Shors and Matzel, 1997). In the present study, blockade of SK channels increases excitability, reduces the threshold for hippocampal synaptic plasticity, and facilitates hippocampal memory encoding. Systemically administered apamin crosses the blood–brain barrier (Habermann, 1984), and high densities of apamin-sensitive SK channels are present in limbic regions, including the hippocampus (Mourre et al., 1987; Gehlert and Gackenheimer, 1993; Stocker and Pedarzani, 2000). Apamin-treated mice acquired a spatial memory for the water maze platform location after just four training trials (minimal training), whereas saline-treated mice required as many as 12 trials to demonstrate spatial memory acquisition. In the object recognition task, apamin-treated mice exhibited a significantly stronger test session preference for the novel object than saline-treated mice when limited to 19 sec of sample object exploration (minimal training). The parallel between the reduction of the threshold for synaptic plasticity and the improved memory encoding after minimal spatial or nonspatial training suggests a correlation between the facilitation of the induction of synaptic plasticity and memory encoding. The amount of induced plasticity cannot be equated with the rate of learning, because control and apamin-treated slices showed the same amount of LTP and LTD. However, the rate of learning seems to be dependent on the threshold for the induction of synaptic plasticity.

Previous studies indicate that apamin enhances spatial memory in mice with lesions of the hippocampal formation but have failed to detect an influence of apamin on memory retention in intact mice after extensive training (Ikonen et al., 1998; Ikonen and Riekkinen, 1999). Moreover, it was proposed recently that there are differences in apamin sensitivity between mice and rats, with rats being relatively insensitive to the cognitive effects of apamin (van der Staay et al., 1999). However, this claim is not substantiated by recent findings. Independent laboratories have demonstrated that in rats, apamin enhances the induction of synaptic plasticity (Behnisch and Reymann, 1998; Norris et al., 1998) and facilitates nonspatial memory (Deschaux et al., 1997; Fournier et al., 2001). Our data suggest that apamin exerts its influence on an early stage of memory encoding, an effect that may not have been detected given 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 previous reports of the effects of apamin in mice and rats (Ikonen et al., 1998; Ikonen and Riekkinen, 1999; van der Staay et al., 1999).

From our behavioral data, no distinction can be made between an effect of apamin that leads to enhanced memory formation and that of an enhanced processing of the sensory input that precedes the formation of memory. However, the results suggest that it is unlikely that the enhancing effects of apamin are a consequence of sensory, motor, or attentional influences. If apamin was to influence sensory or attentional mechanisms, then apamin treatment would have enhanced object memory retention in both groups of mice, those limited to 19 sec of sample exploration as well as those allowed 38 sec of sample exploration. The beneficial

effect of apamin on spatial memory encoding in the Morris water maze cannot be attributed to enhanced motor function, because no differences in swim speed between apamin- and saline-treated mice were observed.

Collectively, the data from electrophysiological and behavioral studies indicate that blockade of SK channels by apamin increases excitability, shifts the threshold for the induction of synaptic plasticity, and facilitates hippocampal-dependent memory. The behavioral significance of this apamin-induced increase in excitability is to facilitate the processing of to-be-remembered information. The behavioral studies do not indicate whether the apamin-mediated enhancement in memory is caused by a facilitation of the induction of LTP or LTD. However, the shift in the threshold for synaptic plasticity produced by apamin could represent a mechanism for ensuring that there is coincident stimulation of hippocampal NMDARs leading to an enhancement of synaptic efficacy during the initial stages of learning. Learning-induced reduction of the AHP has been shown to underlie learning and memory in other behavioral paradigms. A potentiation of EPSPs and a reduction in the mAHP and sAHP currents is associated with classical conditioning of the eye-blink response in rabbits (Disterhoft et al., 1988; LoTurco et al., 1988; Coulter et al., 1989) and with olfactory operant conditioning in rats (Saar et al., 1998). Single-unit recording studies of hippocampal neurons from behaving rabbits during eye-blink conditioning trials have revealed increases in neuronal firing rates that are specific to learning (Berger et al., 1983; McEchron and Disterhoft, 1999). Therefore, the AHP is a negative regulator of learning, and reduction of the AHP by apamin appears to facilitate learning and memory. Together with the results presented here, it appears that apamin-sensitive SK channels represent a neural mechanism capable of regulating hippocampal-dependent memory.

REFERENCES

Alford S, Frenguelli BG, Schofield JG, Collingridge GL (1993) Characterization of Ca^{2+} signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. J Physiol (Lond) 469:693–716.

Andreasen M, Lambert JD (1995) The excitability of CA1 pyramidal cell dendrites is modulated by a local Ca^{2+}-dependent K^+-conductance. Brain Res 698:193–203.

Artola A, Singer W (1993) Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. Trends Neurosci 16:480–487.

Bear MF (1995) Mechanism for a sliding synaptic modification threshold. Neuron 15:1–4.

Behnisch T, Reymann KG (1998) Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro. Neurosci Lett 253:91–94.

Berger TW, Rinaldi PC, Weisz DJ, Thompson RF (1983) Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. J Neurophysiol 50:1197–1219.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2:32–48.

Blatz AL, Magleby KL (1986) Single apamin-blocked Ca-activated K^+ channels of small conductance in cultured rat skeletal muscle. Nature 323:718–720.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Brocher S, Artola A, Singer W (1992) Intracellular injection of Ca^{2+} chelators blocks induction of long-term depression in rat visual cortex. Proc Natl Acad Sci USA 89:123–127.

Cangiano L, Wallen P, Grillner S (2002) Role of apamin-sensitive K_{Ca} channels for reticulospinal synaptic transmission to motoneuron and for the afterhyperpolarization. J Neurophysiol 88:289–299.

Cho YH, Friedman E, Silva AJ (1999) Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. Behav Brain Res 98:77–87.

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. J Neurosci 20:8853–8860.

Coulter DA, LoTurco JJ, Kubota M, Disterhoft JF, Moore JW, Alkon DL (1989) Classical conditioning reduces amplitude and duration of calcium-dependent afterhyperpolarization in rabbit hippocampal pyramidal cells. J Neurophysiol 61:971–981.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC (1996) Ca^{2+} signaling requirements for long-term depression in the hippocampus. Neuron 16:825–833.

Deschaux O, Bizot JC, Goyffon M (1997) Apamin improves learning in an object recognition task in rats. Neurosci Lett 222:159–162.

Disterhoft JF, Golden DT, Read HL, Coulter DA, Alkon DL (1988) AHP reductions in rabbit hippocampal neurons during conditioning correlate with acquisition of the learned response. Brain Res 462:118–125.

Dudek SM, Bear MF (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J Neurosci 13:2910–2918.

Foster TC (1999) Involvement of hippocampal synaptic plasticity in age-related memory decline. Brain Res Brain Res Rev 30:236–249.

Fournier C, Kourrich S, Soumireu-Mourat B, Mourre C (2001) Apamin improves reference memory but not procedural memory in rats by blocking small conductance Ca^{2+}-activated K^+ channels in an olfactory discrimination task. Behav Brain Res 121:81–93.

Gallagher M, Burwell R, Burchinal M (1993) Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav Neurosci 107:618–626.

Garcia ML, Galvez A, Garcia-Calvo M, King VF, Vazquez J, Kaczorowski GJ (1991) Use of toxins to study potassium channels. J Bioenerg Biomembr 23:615–646.

Gehlert DR, Gackenheimer SL (1993) Comparison of the distribution of binding sites for the potassium channel ligands [^{125}I]apamin, [^{125}I]charybdotoxin and [^{125}I]iodoglyburide in the rat brain. Neuroscience 52:191–205.

Habermann E (1984) Apamin. Pharmacol Ther 25:255–270.

Holscher C (1997) Long-term potentiation: a good model for learning and memory? Prog Neuropsychopharmacol Biol Psychiatry 21:47–68.

Ikonen S, Riekkinen Jr P (1999) Effects of apamin on memory processing of hippocampal-lesioned mice. Eur J Pharmacol 382:151–156.

Ikonen S, Schmidt B, Riekkinen Jr P (1998) Apamin improves spatial navigation in medial septal-lesioned mice. Eur J Pharmacol 347:13–21.

Jeffery KJ (1997) LTP and spatial learning–where to next? Hippocampus 7:95–110.

Katz B, Miledi R (1968) The role of calcium in neuromuscular facilitation. J Physiol (Lond) 195:481–492.

Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J, Adelman JP (1996) Small-conductance, calcium-activated potassium channels from mammalian brain. Science 273:1709–1714.

Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons. J Neurosci 20:1791–1799.

Lancaster B, Nicoll RA (1987) Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. J Physiol (Lond) 389:187–203.

Lancaster B, Hu H, Ramakers GM, Storm JF (2001) Interaction between synaptic excitation and slow afterhyperpolarization current in rat hippocampal pyramidal cells. J Physiol (Lond) 536:809–823.

Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. Proc Natl Acad Sci USA 86:9574–9578.

LoTurco JL, Coulter DA, Alkon DL (1988) Enhancement of synaptic potentials in rabbit CA1 pyramidal neurons following classical conditioning. Proc Natl Acad Sci USA 85:1672–1676.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305:719–721.

Magee JC, Johnston D (1995) Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. Science 268:301–304.

Malenka RC, Nicoll RA (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. Trends Neurosci 16:521–527.

Malenka RC, Nicoll RA (1999) Long-term potentiation–a decade of progress? Science 285:1870–1874.

Malenka RC, Lancaster B, Zucker RS (1992) Temporal limits on the rise in postsynaptic calcium required for the induction of long-term potentiation. Neuron 9:121–128.

Martin ED, Araque A, Buno W (2001) Synaptic regulation of the slow Ca^{2+}-activated K^+ current in hippocampal CA1 pyramidal neurons: implication in epileptogenesis. J Neurophysiol 86:2878–2886.

Mayer ML, MacDermott AB, Westbrook GL, Smith SJ, Barker JL (1987) Agonist- and voltage-gated calcium entry in cultured mouse spinal cord neurons under voltage clamp measured using arsenazo III. J Neurosci 7:3230–3244.