THE PROTECTIVE EFFECTS OF CHRONIC CAFFEINE TREATMENT ON THE COGNITIVE FUNCTION AND SYNAPTIC PLASTICITY IN ACUTE SLEEP DEPRIVATION

A Dissertation Presented to the
Faculty of the Department of Pharmacological and Pharmaceutical Sciences
College of Pharmacy, University of Houston

In Partial Fulfillment
of the Requirement for the Degree
Doctor of Philosophy

By
Ibrahim A. Alhaider
April, 2010

THE PROTECTIVE EFFECTS OF CHRONIC CAFFEINE TREATMENT ON THE COGNITIVE FUNCTION AND SYNAPTIC PLASTICITY IN ACUTE SLEEP DEPRIVATION

Ibrahim A. Alhaider

Approved by Dissertation Committee:

Dr. Karim A. Alkadhi, Advisor

Dr. Vincent Lau, Committee Member

Dr. Tahir Hussain, Committee Member

Dr. J. Leigh Leasure, Committee Member

Dr. Abdulaziz Aleisa, Committee Member

Dr. Lamar Pritchard, Dean
College of Pharmacy

April, 2010

ACKNOWLEDGMENT

First of all, praise and thanks to Allah for providing me the blessings to complete my project.

There are many people without whom this work might never have been completed, and I would like to give them special appreciation.

I would like to express my deep and sincere gratitude to my advisor, Dr. Karim Alkadhi. I have been providential to have an advisor who afforded me the freedom to explore on my own, and, at the same time, the direction to retrieve when my steps hesitated.

I offer my regards to my committee members; Dr. Vincent Lau, Dr. Tahir Hussain, Dr. J Leigh Leasure, and Dr. Abdulaziz Alesia, who were more than generous with their proficiency and precious time.

I am also grateful to my colleagues in the lab, Dr. Trinh Tran, Dr. Munder Zagaar, Dr. Marisa Srivareerat, and Dr. Karem Alzoubi, for their various forms of support and teaching during my graduate study.

Though only my name appears on the cover of this dissertation, I owe my deepest gratitude and thanks to my wife, Reem, who has valuably facilitated its production.

I am heartily thankful to my son, Abdulrahman, and my daughter, Shahd, who made the completion of this project a pleasant experience. I would like to thank my brothers and sisters, whose support was influential in keeping me motivated during my research.

Lastly, and most importantly, no expressions could describe my gratitude to my parents, Abdulrahman and Norh, who continues to learn and raise and, who has been a source of inspiration to me throughout my life.

THE PROTECTIVE EFFECTS OF CHRONIC CAFFEINE TREATMENT ON THE COGNITIVE FUNCTION AND SYNAPTIC PLASTICITY IN ACUTE SLEEP DEPRIVATION

An Abstract of
A Dissertation Presented to the
Faculty of the Department of Pharmacological and Pharmaceutical Sciences
College of Pharmacy, University of Houston

In Partial Fulfillment
of the Requirement for the Degree
Doctor of Philosophy

By
Ibrahim A. Alhaider
April, 2010

ABSTRACT:

Study objectives: Accumulating evidence has shown that caffeine and sleep deprivation have opposing effects on learning and memory; therefore, this study was undertaken to provide a detailed account of the effect of chronic, low-dose caffeine treatment on the deleterious effects of sleep loss on hippocampus-dependent learning and memory.

Experimental design: We investigated the effects of chronic (4 weeks) caffeine treatment (0.3 g/l in drinking water) on memory impairment in acutely (24 hr) sleep-deprived rats. Sleep deprivation was induced using the modified multiple platform model. The effects of caffeine on sleep deprivation-induced hippocampus-dependent learning and memory deficits were studied using three approaches: learning and memory performance in the radial arm water maze task; electrophysiological recordings in the Cornu Ammonis (CA1) and dentate gyrus (DG) regions of the hippocampus; and western blot analysis to measure the levels of memory- and synaptic plasticity-related signaling molecules.

Results: Our results showed that chronic caffeine treatment prevented impairment of hippocampus-dependent learning, short-term memory and early phase- long-term potentiation (E-LTP) of the CA1 and DG areas in the sleep-deprived rats. In correlation, caffeine treatment prevented a sleep deprivation-associated decrease in the basal levels of phosphorylated calcium/calmodulin-dependent protein kinase II (P-CaMKII) and

brain-derived neurotrophic factor (BDNF). In addition, caffeine treatment of sleep-deprived rats increased the levels of P-CaMKII during the expression of E-LTP.

The results also showed that chronic caffeine treatment prevented the impairment of long-term memory and late phase-LTP (L-LTP) in the CA1 and DG regions of the sleep-deprived rats. Additionally, caffeine treatment prevented a sleep deprivation-associated decrease in the basal levels of the phosphorylated cAMP response element binding protein (P-CREB) as well as total CREB. Treating sleep-deprived rats chronically with caffeine enables multiple high frequency stimulation to increase the levels of P-CREB during L-LTP expression.

Conclusions: The results suggest that long-term use of a low dose of caffeine protects against the harmful changes in the basal levels of P-CaMKII, P-CREB and BDNF associated with sleep deprivation and as a result contributes to the revival of hippocampus-dependent learning and memory as well as LTP in the CA1 and DG regions.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

A_1	adenosine A_1
A_{2a}	adenosine A_{2a}
AB	angular bundle
AC	adenylyl cyclase
AD	Alzheimer's disease
AMPA	amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AP	anterior posterior
BCA	bicinchoninic acid
BDNF	brain-derived neurotrophic factor
CA1	Cornu Ammonis 1
Ca^{2+}	calcium
CaMKII	calcium/calmodulin-dependent protein kinase II
CaMKIV	calcium/calmodulin-dependent protein kinase IV
cAMP	cyclic adenosine mono-phosphate
CREB	cAMP response element binding protein
D	dorsal
DAG	diacylglycerol
IP_3	inositol tri-phosphate

DG	dentate gyrus
ECL	chemiluminescence
EEG	electroencephalogram
E-LTP	early phase-LTP
fEPSP	field excitatory post-synaptic potential
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
HFS	high frequency stimulation
HTH	high throughout
i/o	input/output
L	lateral
L-LTP	late phase-LTP
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MHFS	multiple high frequency stimulation
MWM	Morris water maze
NMDA	N-methyl-D-aspartate
NREM	non-rapid eye movement
p-CaMKII	phosphorylated-calcium/calmodulin kinase II

p-CREB	phosphorylated-cAMP response element binding protein
PD	Parkinson's disease
PDE	phosphodiestrase
ERK	extracellular signal-regulated kinase 2
PP1	protein phosphatase 1
PP2B	calcineurin
pSpike	population spike
PVDF	polyvinylidine fluoride
RAM	radial arm maze
RAWM	radial arm water maze
REM	rapid eye movement
S	stimulated
SD	sleep deprivation
SEM	standard error of the mean
SWS	slow wave sleep
T	time
t-CaMKII	total-calcium calmodulin kinase II
t-CREB	total-cAMP response element binding protein
TrkB/PLCγ	tyrosine kinase B receptor/phospholipase Cγ

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

1- INTRODUCTION AND STATEMENT OF THE PROBLEM

The National Sleep Foundation maintains that seven to eight hours (hrs) of sleep are necessary for optimal cognitive performance. Deficits in cognitive function as a consequence of sleep loss are experienced universally in modern civilizations. As of 2001, statistics indicated that 38% of adult Americans reportedly slept an average of eight hrs. This figure had dropped to 28% in 2009 (National Sleep Foundation, 2009). Even though research has led to an increase in our knowledge of underlying physiology, we still have little understanding of the impact that environmental factors, such as caffeine consumption, have on memory decline associated with sleep deprivation.

Caffeine, a commonly used central nervous system stimulant (Nicholson and Stone, 1980; Nehlig et al., 1992; Ferre, 2008), is implicated in the modulation of the learning and memory function (Kopf et al., 1999; Dall'Igna et al., 2004). Several lines of evidence have shown that caffeine enhances the cognitive function in both clinical studies and experimental animal models (Lieberman et al., 1987; Durlach, 1998; Angelucci et al., 2002). Additionally, caffeine administration alleviates memory impairment resulting from a variety of animal models of brain disorders including Alzheimer's disease (Arendash et al., 2006; Dall'Igna et al., 2007; Arendash et al., 2009), Parkinson's disease (Gevaerd et al., 2001), attention deficit hyperactivity disorder (Prediger et al., 2005), age-related cognitive decline (Riedel and Jolles, 1996), and

scopolamine-induced amnesia (Riedel et al., 1995). The beneficial effects of caffeine administration on memory are attributed to its action as a non-selective adenosine receptor antagonist (Nehlig et al., 1992; Takahashi et al., 2008; Pires et al., 2009). Furthermore, caffeine has been reported to influence synaptic plasticity. In hippocampal slices, caffeine induces long-term potentiation (LTP) in the Schaffer collaterals-CA1 pyramidal neuron synapses (Martin and Buno, 2003).

Sleep plays a pivotal role in normal biological functions. Although its exact functions remain elusive, sleep may be a fundamental contributor to memory consolidation (Diekelmann and Born, 2010, ; Blissitt et al., 2001). The influence of sleep on learning and memory can be evaluated by employing sleep deprivation as a model. There is a large body of evidence showing a strong correlation between sleep deprivation and memory impairment in humans and animals (Polzella, 1975b; Kim et al., 2005; Ferrara et al., 2008). For example, in the hippocampus, sleep deprivation prior to learning reduces learning ability and impairs memory (Yang et al., 2008; Hagewoud et al., 2009), whereas sleep deprivation following learning impairs memory formation (Harrison and Horne, 2000; Li et al., 2009; Wang et al., 2009). Evidence indicates that while sleep loss causes a significant decrease in hippocampal activity (Yoo et al., 2007), the hippocampus becomes more active when the subjects are permitted to sleep after a learning task (Gais et al., 2007).

The activity of the neural networks within the hippocampus, a well-known structure involved in memory of locations or spatial memory, can undergo changes in the strength of synaptic transmission as a result of repetitive stimulation (Shapiro and Eichenbaum, 1999; Burgess et al., 2002). These changes, known as synaptic plasticity, are required for learning and memory processes. Two main phases of long-term potentiation have been studied extensively, the early-phase (E-LTP), which depends mainly on calcium/calmodulin-dependent protein kinase II (CaMKII) phosphorylation, and the late-phase (L-LTP), which requires protein synthesis through activation of transcription factors such as cAMP response element binding protein (CREB). The two phases are widely accepted as hypothetical models for short-term and long-term memory, respectively (Malenka and Nicoll, 1999; Malenka and Bear, 2004). Accordingly, earlier studies have shown that sleep deprivation impairs LTP in the CA1 and DG areas of the hippocampus (Kim et al., 2005; Marks and Wayner, 2005).

The molecular mechanisms underlying the expression of E-LTP and L-LTP have been studied in detail. For E-LTP, upon high-frequency stimulation (HFS), glutamate released from presynaptic terminals binds to the glutamatergic N-methyl-D-aspartate (NMDA) receptors on the post-synaptic membrane, causing a large influx of Ca^{2+} (Malenka et al., 1988), which binds to calmodulin, forming a calcium-calmodulin complex that activates CaMKII. The activated CaMKII is widely accepted as the regulator

of E-LTP (Fukunaga et al., 1996; Miyamoto and Fukunaga, 1996; Barria et al., 1997a; Fukunaga and Miyamoto, 2000). In contrast, phosphatases, such as calcineurin, are responsible for the dephosphorylation of active CaMKII (Wang and Kelly, 1996). An additional pathway through which CaMKII can be activated is through the tyrosine kinase B receptor/phospholipase Cγ (TrkB/PLCγ) pathway in the hippocampus by the binding of brain-derived neurotrophic factor (BDNF) to the tyrosine kinase receptors to release Ca^{2+} from intracellular stores (Blanquet and Lamour, 1997; Minichiello et al., 2002). Upon multiple high-frequency stimulation (MHFS), large and highly localized Ca^{2+} activates calcium-calmodulin-dependent protein kinase IV (CaMKIV), which can directly phosphorylate CREB, and as a result activates multiple genes required for L-LTP generation (Bito et al., 1996; Tokuda et al., 1997). It has been reported that gene expression of CaMKII, CREB and BDNF, is reduced after 8 hrs of sleep deprivation (Guzman-Marin et al., 2006).

Although the pharmacological effects of caffeine have been extensively studied, the long-term effects of this drug on sleep deprivation-induced learning and memory impairment in the hippocampus have not been fully investigated. This project was designed to imitate the common occurrence of long-term caffeine consumption and the occasion of sleep deprivation. Based on the known impact of caffeine and sleep deprivation on memory, we hypothesized that chronic caffeine intake prevents

hippocampus dependent learning and memory impairment associated with sleep loss of 24 hr.

In the current project, we tested the following three hypotheses: (1) chronic caffeine treatment prevents 24 hr sleep deprivation-induced impairment of hippocampus-dependent learning and memory, (2) chronic caffeine treatment prevents sleep deprivation-induced E-LTP and L-LTP impairment in the hippocampal CA1 and DG regions, and (3) antagonism of sleep deprivation-induced effects on E-LTP and L-LTP by long-term caffeine intake is the result of caffeine-induced changes in the levels of signaling molecules including CaMKII, BDNF, CREB and/or calcineurin after 24 hr of sleep deprivation in the CA1 and DG areas.

These hypotheses were tested with three different approaches. Behavioral experiments were performed using a radial arm water maze (RAWM) to determine the effect of caffeine on sleep deprivation-induced impairment of learning and memory. Then, in a new set of animals, electrophysiological recordings were carried out in the hippocampus of urethane-anesthetized rats to determine the influence of caffeine treatment on sleep deprivation–induced impairment of E-LTP and L-LTP in the CA1 and DG areas of hippocampus. Finally, western blot analysis was used to determine the impact of caffeine on sleep deprivation-induced changes in the levels of signaling molecules essential for memory and synaptic plasticity. The results of this study will be

an important contribution to the understanding of the mechanism(s) by which caffeine prevents memory impairment in an animal model of sleep deprivation.

2. LITERATURE SURVEY

2.1. Memory

Learning and memory are distinct cognitive processes for acquiring and retaining information about the individual's environment (Bailey et al., 1996; Lynch, 2004). Based on the contribution of various brain structures and neural circuits during assessments of free recall and recognition, memory can be divided into two major categories (Diekelmann and Born, 2010 ; Schacter et al., 1992). Implicit, or non-declarative memory, is recall of information for tasks and skills that result from repeated practice and is not necessarily for conscious remembrance, e.g., driving a car. Implicit memory is believed to rely on the striatum and cerebellum, although current reports also suggest a function for hippocampus in implicit memory (Diekelmann and Born, 2010 ; Heindel et al., 1989; Packard et al., 1989; Bailey et al., 1996; Barco et al., 2006). On the other hand, explicit, or declarative memory, involves conscious and intentional recall of faces, spatial arrangements, objects, and events. This type of memory relies on the hippocampus and associated medial temporal lobe structures, along with neocortical areas for long-term storage (Diekelmann and Born, 2010 ; Cohen and Squire, 1980). The memory for implicit and explicit categories can be graded, and its duration classified into two distinct forms: short-term memory, which lasts for a few minutes and up to two hrs; and long-term

memory, which persists for several hours, days, weeks, and in some situations, even a lifetime (Suarez et al., 2010 ; Glanzer, 1971).

2.2. The hippocampus and memory

The hippocampus, a bilateral limbic structure located beneath the cerebral cortex, functions as a temporary storage depot for memory (Meissner, 1966; Teyler and DiScenna, 1985, 1986). From human and animal studies, much evidence supports the essential role of the hippocampus in learning and memory. The most direct and convincing evidence has come from the famous case of a patient named Henry Gustav Molaison (H.M), who had suffered from an untreatable type of seizure (Winters et al., 2010 ; Miller et al., 2009). He underwent surgery to remove part of his medial temporal lobe including hippocampal formation, for relief epileptic seizures. Following the surgery, H.M developed severe anterograde amnesia in which he lost the ability to form new memory, but retained old memory (Scoville and Milner, 1957). In another case, a patient known as R.B. was diagnosed with severe anterograde amnesia associated with ischemic damage as a result of open heart surgery. Examination of R.B.'s brain after his death revealed bilateral lesions that were localized in area CA1 of the hippocampus (Zola-Morgan et al., 1986). This clearly showed that damage of the CA1 area is enough to generate memory deficit. Additionally, destruction of the hippocampus in animals

caused severe deficit of spatial memory in the water maze (Morris et al., 1982) and radial arm maze (Olton, 1977; Olton and Papas, 1979).

2.3. Anatomy of the hippocampus

Anatomically, the hippocampus is arranged in a lamellar manner to receive highly processed information from various neocortical areas. The top part of the hippocampus is called the “dorsal” or “septal” hippocampus, while the bottom part is known as the “ventral” or “temporal” hippocampus. A cross section of the hippocampus discloses its internal laminar structures as two interlocking “Cs” (fig. 1). The first “C” corresponds to Ammon’s Horn or Cornu Ammonis (CA1-CA4 subfields), in which pyramidal cells form the principal cell layer. The second “C” corresponds to the dentate gyrus (DG) area, in which granule cells form the principal cell layer. Both CA1 and DG areas are involved in the intrinsic flow of information within the hippocampus (Amaral and Witter, 1989; Witter et al., 1989; Xavier et al., 1999).

The hippocampus is characterized by a tri-synaptic circuit, through which the flow of information within the hippocampus proceeds uni-directionally (fig. 1). The fibers from the entorhinal cortex, forming the perforant path, synapse on the granule cells in the DG area. Next, the mossy fiber pathway projects from the axons of the granule cells in the DG area to the pyramidal cells in the CA3 area. Finally, in the Schaffer collateral pathway, the axons of the pyramidal neurons in the CA3 synapse on

the pyramidal cells in the CA1 area of the hippocampus. Additionally, the axons of the CA3 pyramidal cells project on the contralateral hippocampus (the commissural pathway) (Amaral and Witter, 1989; Witter et al., 1989). Repetitive stimulation of any of these pre-synaptic pathways evokes long-lasting changes in synaptic responses of the target hippocampal neurons of that particular pathway.

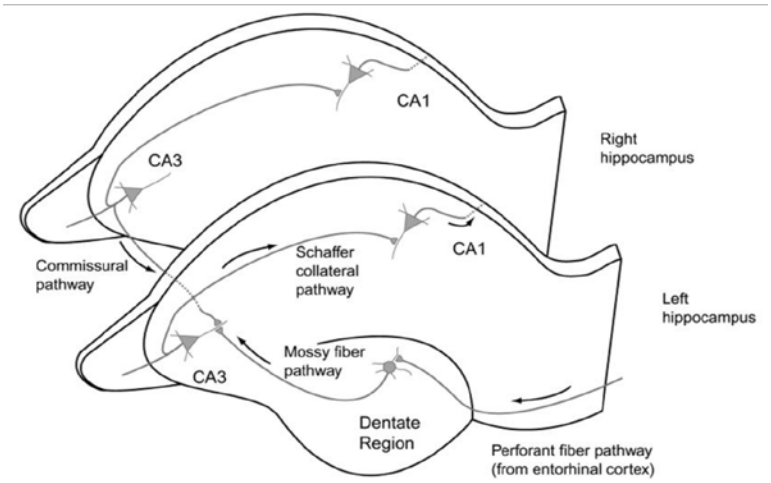


Figure 1: Trisynaptic pathways of the hippocampus. Perforant fibers start from the entorhinal cortex and synapse on the granule cells of the dentate gyrus. Mossy fiber axons of dentate gyrus granule cells synapse on CA3 pyramidal cells. Schaffer collaterals of CA3 pyramidal cells synapse on CA1 pyramidal cells. CA3 pyramidal cells, through the commissural pathway, send projections to the contralateral hippocampus (Kandel, 2001b).

2.4. Memory models/tests

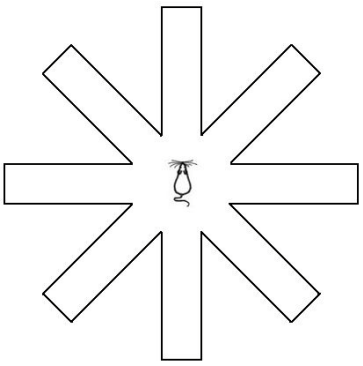
Several models have been established to test various types of memory tasks in order to determine the brain areas involved in certain cognitive processes.

2.4.1 Animal mazes

The maze is the most commonly used approach to test spatial learning and memory (memory of locations; hippocampus-dependent memory) in laboratory animals. Using spatial cues, animals must learn the location of certain desirable goals like food or safety. In general, mazes come in different sizes and shapes ranging from a featureless area, such as a water maze, to the predetermined pathways, such as a radial arm water maze. Various types of mazes have been established and successfully used to test learning and memory. For example, in the radial arm maze, damage to the hippocampus impairs spatial memory (Olton, 1977; Olton and Papas, 1979). In the following, I will discuss the most popular types of mazes.

2.4.1.1. The radial arm maze (RAM)

In 1976, the RAM was designed by Olton and Samuelson to test spatial learning and memory (Olton, 1987; Dubreuil et al., 2003). The original RAM consisted of eight

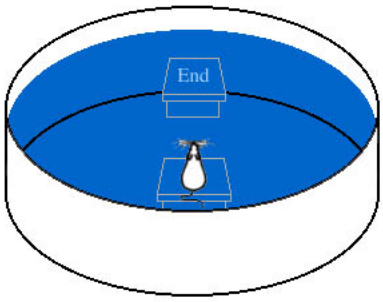


arms radiating from a central location. To test short- and long-term memory, the ends of four of eight arms were baited with food for each daily training trial. The baited arms remained constant throughout the trials. Following food deprivation, each animal was placed in the center of the maze and permitted to enter different arms. Training

continued until all four food rewards were eaten or until five minutes had passed. The animal should have remembered which arms were visited previously during a particular trial in order to avoid reentering these arms, since food was no longer obtainable. The number of short-term memory errors (entering a baited arm more than once) and long-term memory errors (entering an unbaited arm) were noted. In the RAM task, the rats with lesions in the hippocampus showed memory impairment (Olton, 1977; Olton and Papas, 1979). Some limitations of RAM include food deprivation and odors, either from the food at the end of the rewarded arms, or from other animals. However, the RAM has the advantage of being able to simultaneously evaluate both short-term memory and long-term memory.

2.4.1.2. The Morris water maze (MWM)

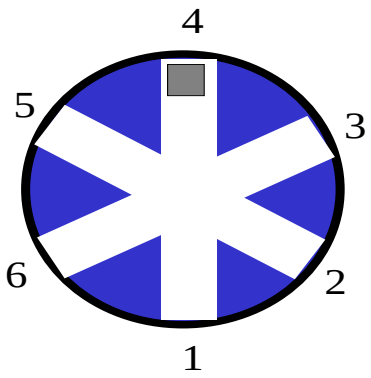
The “water maze” or "MWM” was created by Richard Morris in 1981, and developed by David Olton (1982). The MWM is made up of a large round tub of opaque water (made white with powdered milk) and a hidden platform, located in the centre of one of four quadrants. The MWM is commonly used to test spatial memory by using visuospatial signs to enable swimming animals to find a hidden platform to stand on (Morris, 1984). The animal is released from one of four cardinal



points around the maze. The time spent by animals to locate the hidden platform should decrease as the trial progresses. This measured time reflects the speed of learning. To test spatial memory, the hidden platform is removed and the time spent in the former region of the platform is recorded. The MWM eliminates some of the disadvantages of the RAM including food deprivation and olfactory guiding cues during training. However, swimming an appropriate fixed distance around the sides of the MWM as a strategy to find the hidden platform is one of the main disadvantages of MWM (Hodges, 1996). Hippocampal lesions have been shown to impair spatial memory in the MWM.

2.4.1.3. The radial arm water maze (RAWM)

The RAWM, developed by Buresova et al (1985), is a combination of both- RAM and MWM. The maze looks like a 6-arm RAM inserted into a MWM. Detailed procedure of the RAWM is discussed on page 40.



In the current study, we used the RAWM to evaluate spatial learning and memory performance. The RAWM maintains the advantage of simultaneously evaluating short-term memory and long-term memory. Additionally, the RAWM removes the disadvantages of food deprivation

and odor associated with the RAM method and eliminates the issue of swimming around the pool that is associated with the MWM method (Shukitt-Hale et al., 2004).

2.4.2. Electrophysiological studies

Hippocampal neurons communicate with each other to form networks, which are arranged in functionally related circuits (McIntosh, 2000). Function at the behavioral level is an outcome of the integrated function of these neuronal systems rather than simply the result of individual neurons. Although electrophysiology studies are critical for understanding details of these processes, they are unable to incorporate such findings to ultimately and completely explain cognitive functions. Therefore, an understanding of hippocampal electrophysiology is essential to assess the functional integrity of neural pathways on the cellular level.

The distinct anatomical and histological structure of the hippocampus allows electrophysiological experiments to be performed *in vitro* (hippocampal slices) and *in vivo* (anesthetized animals). Studies of brain slices are informative because they are easy to manipulate. For example, pharmacological manipulation can be carried out easily and in a quantitative way, and the direct visualization of definite hippocampal subfields affords precise location of electrodes. However, the interpretations from the *in vitro*

studies may be affected by disrupted circuitry, loss of neurotransmitters, and temperature variations.

2.5. Memory and synaptic plasticity

Although LTP is widely considered as a cellular model of learning and memory, there is no clear confirmation to demonstrate that LTP is the electrophysiological basis of memory. However, there are shared characteristics between memory and LTP, which provide evidence for this correlation. Some of these characteristics are: (1) LTP is easily expressed in the major synaptic pathways of the hippocampus, (2) like memory, LTP relies on alterations in the synaptic efficacy of networks of neurons rather than in an individual neuron, and (3) there are diverse phases of LTP (e.g., E-LTP and L-LTP) with different durations and molecular cascades (Sweatt, , 1999). These phases could be correlated with different types of memory (short- and long-term memory).

Electrophysiological experiments provide a large body of evidence that establishes a link between LTP and memory. For example, an increase in glutamate release and an enhancement of EPSPs have been demonstrated in the perforant pathway of the hippocampus during spatial learning tasks as well as in LTP (Richter-Levin et al., 1995). Additionally, since LTP is a saturable phenomenon, many investigators have hypothesized that if learning and memory mediate LTP-like changes, then saturation of LTP should prevent the acquisition of new learning and memory. Indeed,

saturation of LTP bas been observed to impair spatial learning and memory (McNaughton et al., 1986; Moser et al., 1998), which supports the correlation between spatial memory and LTP.

In addition to the electrophysiological experiments, pharmacological and genetic treatments confirm the association between memory and LTP by showing that both memory and LTP depend on similar molecular cascades to be stored and expressed, respectively. For instance, N-methyl-D-aspartate (NMDA) glutamate receptor activation is required for both spatial memory formation and induction of LTP. When the postsynaptic membrane is depolarized, the consequent activation of the ionotropic NMDA receptors by glutamate causes Ca^{2+}influx. Antagonizing NMDA receptors by 2-amino-5-phosphonopentanoate impairs spatial learning and memory and prevents LTP (Morris et al., 1986). Additionally, mice that lacked NMDA receptor GluN1 (formerly known as NR1) subunit in area CA1 (Tsien et al., 1996) or the NMDA receptor GluN2A (formerly known as NR2A) subunit (Sakimura et al., 1995) causes spatial learning and memory and LTP deficits. Therefore, pharmacological and genetic studies confirm the correlation between spatial memory and hippocampal-LTP.

Another electrophysiological phenomenon that is thought to be associated with learning and memory is long-term depression (LTD), which is an activity-dependent decline of synaptic strength. Induction of LTD has been accomplished, with several

induction protocols, in many parts of the brain that are known to support LTP (e.g. CA1 region). LTD can be induced by low frequency stimulation in hippocampal slices of young animals only (Fujii et al., 1991; O'Dell and Kandel, 1994), and by paired pulse facilitation in anesthetized or freely moving adult animals (Doyere et al., 1996; Thiels et al., 1996; Aleisa et al., 2006b; Alzoubi et al., 2007b).

2.6. Hippocampal formation and long-term potentiation

Two major phases of hippocampal LTP have been identified in the CA1 and the DG areas; early (E-LTP) and late (L-LTP), both of which require NMDA receptors activation by glutamate (Sweatt, , 1999). In E-LTP, a single train of HFS generates an increase in synaptic strength that lasts more than 30 min and up to three hrs. Conversely, late (L-LTP), requires four trains of HFS to generate an increase in synaptic strength that lasts more than three hr. Each phase of LTP is activated by different protein kinases. Furthermore, the expression of L-LTP requires gene expression and protein synthesis (Sweatt et al., 1999).

2.6.1. Expression of E-LTP and L-LTP

The expression of LTP is initiated by tetanic stimulation of the presynaptic membrane, which enhances glutamate release. The released glutamate binds to postsynaptic NMDA receptors to allow the influx of Ca^{2+} (Ascher and Nowak, 1988) (fig.

2). This rapid and large transient increase in Ca^{2+} concentration activates CaMKII, which is believed to play a crucial role in the generation and maintenance of E-LTP in the hippocampus (Malenka et al., 1989; Pettit et al., 1994; Thomas et al., 1994; Lledo et al., 1995; Giese et al., 1998). The autophosphorylation feature of CaMKII makes it constitutively active, even in the absence of Ca^{2+}, until protein phosphatases such as calcineurin dephosphorylate P-CaMKII. The constitutively active P-CaMKII phosphorylates and activates amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (fig. 2), which are important for the expression of E-LTP (Fukunaga et al., 1996; Nayak et al., 1996; Wang and Kelly, 1996; Fukunaga and Miyamoto, 2000).

Multiple tetanic stimulation induces L-LTP (fig. 3), which causes massive and focused calcium influx into the post-synaptic membrane. Late (L)-LTP requires activation of calcium/calmodulin kinase IV (CaMKIV) and mitogen-activated protein kinase (MAPK) to phosphorylate cAMP response element-binding protein (CREB) (fig. 3). Active (phosphorylated) CREB stimulates the expression of target genes including BDNF (Nguyen and Kandel, 1996; Bolshakov et al., 1997; Lu et al., 1999b; Kandel, 2001a; Barco et al., 2002; Barco et al., 2005).

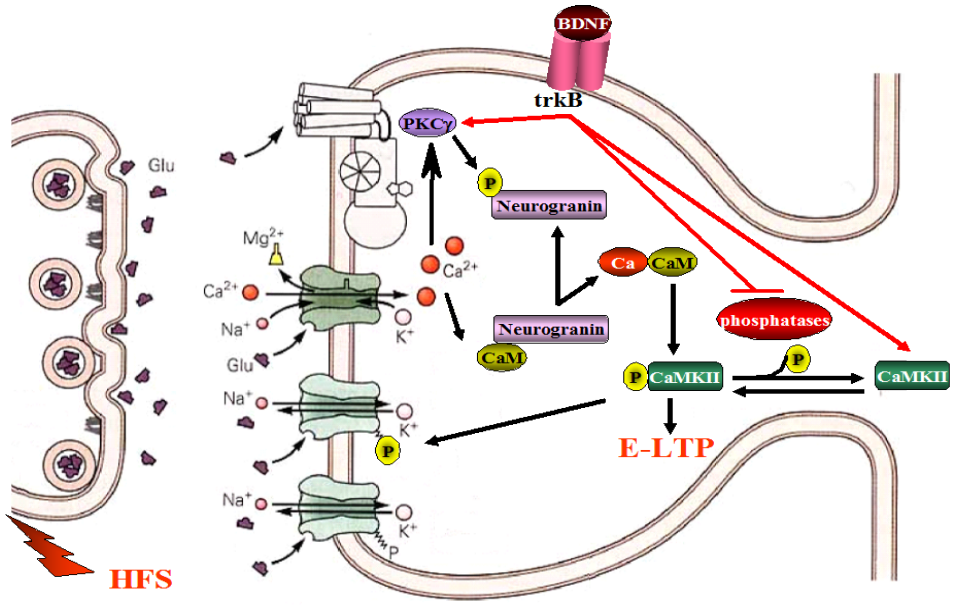


Figure 2: E-LTP scheme. At normal intracellular calcium concentrations $[Ca^{2+}]i$, neurogranin forms a stable complex with calmodulin (CaM). However, high $[Ca^{2+}]i$ after HFS will bind to calmodulin forming calcium-calmodulin complex (Ca/CaM) which activates CaMKII. Activated CaMKII (P-CaMKII) maintain its activity for a few hours and has the ability to phosphorylate AMPA-receptors. P-CaMKII is inactivated by protein phosphatases. Additionally, BDNF enhances activation of CaMKII probably through tyrosine kinase B receptor/phospholipase Cγ pathway (Modified from Kandel and Schwartz 2001).

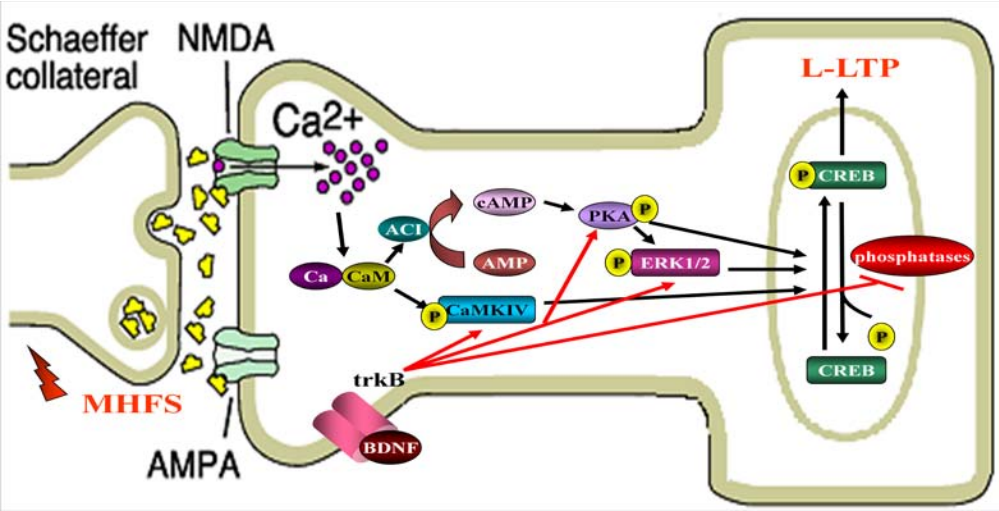


Figure 3: L-LTP scheme. Upon multiple high frequency stimulation (MHFS), generation of Ca-CaM activates ACI-PKA-MAPKp44/42 pathway and CaMKIV, which in turn phosphorylate CREB. Active (phosphorylated) CREB induces the expression of target genes including brain-derived neurotrophic factor. BDNF acting on TrkB prevents activation of phosphatases, and activates CaMKIV and MAPKp44/42 (Modified from Kandel and Schwartz 2001).

2.7. Important subcellular molecules involved in LTP and memory

The finding that the increase in Ca^{2+} concentration in the postsynaptic membrane is crucial for the expression of E-LTP and L-LTP directs attention to the downstream signaling regulators including CaMKII, calcineurin, BDNF, CREB, and CaMKIV.

2.7.1. Calcium calmodulin-dependent protein kinase II (CaMKII)

CaMKII is essential in the expression of LTP in hippocampus (Malenka et al., 1989; Pettit et al., 1994; Lledo et al., 1995; Giese et al., 1998). Whereas CaMKII inhibitors

prevent the expression of LTP (Malenka et al., 1989; Malinow et al., 1989), CaMKII activators generate synaptic enhancement similar to LTP. It has been shown that sleep deprivation reduces the gene expression of CaMKII, which may explain the mechanism for the sleep deprivation-induced impairment of LTP (Guzman-Marin et al., 2006).

It has been shown that HFS, required to induce E-LTP, increases the phosphorylated and non-phosphorylated CaMKII, in hippocampal slices (Ouyang et al., 1997) and urethane-anesthetized rats (Gerges et al., 2004a). Induction of E-LTP in the Schaffer collateral/CA1 synapse leads to the release of glutamate, which in turn activates postsynaptic glutamate receptors. Activation of NMDA receptors increases the concentration of intracellular Ca^{2+}. This increase in intracellular Ca^{2+}, which is transient, frees calmodulin from the calmodulin-neurogranin complex to form a Ca^{2+}/calmodulin complex. The Ca^{2+}/calmodulin complex binds to and triggers CaMKII autophosphorylation (fig. 2). This rapid autophosphorylation of CaMKII generates a constitutively active CaMKII (P-CaMKII) that phosphorylates AMPA receptors. The phosphorylated form of AMPA receptors is important for the expression of LTP (Fukunaga et al., 1996; Nayak et al., 1996).

2.7.2. Calcineurin (PP2B)

A number of protein phosphatases are present in the hippocampal neurons. These protein phosphatases are responsible for the dephosphorylation and deactivation

processes of previously phosphorylated molecules. Activation of PP2B (calcineurin) by Ca^{2+}/calmodulin leads to the activation of protein phosphatase 1 (PP1), which in turn inactivates P-CaMKII (fig. 2) (Mulkey et al., 1993; Mulkey et al., 1994). It has been shown that over-expression of calcineurin in the hippocampus attenuates hippocampal-dependent memory formation (Mansuy et al., 1998). There are three major types of protein phosphatases in hippocampal neurons: PP1, PP2A and PP2B. Phosphatases PP1 and PP2A are supposed to be effective in dephosphorylation of CaMKII at Thr286, thus reverting its constitutive activity to basal levels. It has been shown that induction of LTP in the hippocampus is associated with a marked decrease in PP2A activity, which results in elevation of CaMKII activity and potentiation of synaptic efficacy (Fukunaga et al., 2000).

2.7.3. Brain-Derived Neurotrophic Factors (BDNF)

BDNF is widely believed to be important in memory and its cellular models. For example, BDNF has been observed to enhance memory (Kiprianova et al., 1999; Alonso et al., 2002) and LTP (Akaneya et al., 1997; Kiprianova et al., 1999). Endogenous BDNF plays an essential role in spatial learning and memory and hippocampal LTP. Studies that used BDNF-knockout mice show impairment in spatial memory (Linnarsson et al., 1997) and hippocampal LTP (Korte et al., 1995; Patterson et al., 1996). Furthermore, the gene expression of BDNF has been reported to be increased in the hippocampus after spatial

learning tasks (Mizuno et al., 2000; Gooney et al., 2002), and after the induction of LTP (Patterson et al., 1992; Castren et al., 1993; Dragunow et al., 1993; Springer et al., 1994; Morimoto et al., 1998). Additionally, numerous studies found an increase of synaptic transmission and a potentiation of the synaptic response, which resembled LTP, after acute treatment of exogenous BDNF in CA1 hippocampal slices (Korte et al., 1995; Figurov et al., 1996) and cultures (Sherwood and Lo, 1999), and following intrahippocampal infusion of BDNF into the DG area of anesthetized rats (Messaoudi et al., 1998). In the hippocampus, sleep-deprived rats showed a reduction in the expression of BDNF mRNA as well as in the basal protein levels of BDNF (Guzman-Marin et al., 2006).

In the hippocampus, BDNF enhances activation of CaMKII and CREB, probably by a mechanism that involves the release of Ca^{2+} from the internal stores through tyrosine kinase B receptor/phospholipase Cγ pathway (Blanquet and Lamour, 1997; Finkbeiner et al., 1997; Minichiello et al., 2002). Brain-derived neurotrophic factor binds to TrkB receptors and activates PLCγ signaling pathway to generate diacylglycerol (DAG) and inositol tri-phosphate (IP_3) and the latter releases Ca^{2+} from cytoplasmic stores.

2.7.4. cAMP response element binding protein (CREB)

CREB is a transcription factor involved in the regulation of CRE-containing genes. Accumulating evidence has shown that expression of L-LTP and long-term memory

requires CREB activation (Yin et al., 1994; Tully, 1997; Abel and Kandel, 1998; Abel et al., 1998). Induction of repressor or activator forms of CREB affects long-term memory negatively or positively, respectively, with no effect on short-term memory (Yin et al., 1994; Yin et al., 1995). In fact, mice with mutation in CREB have defective L-LTP and long-term memory, but normal E-LTP and short-term memory (Bourtchuladze et al., 1994). Intrahippocampal infusion of oligonucleotides that shut down CREB gene expression does not alter short-term spatial memory in rats but affects memory tested two days after the acquisition phase (Guzowski and McGaugh, 1997). Spatial memory tasks enhance CREB phosphorylation in the hippocampus (Colombo et al., 2003) as does L-LTP induction (Bito et al., 1996). Consistent with this evidence, CREB has been reported to be important in the generation of long-term memory and L-LTP (Dash et al., 1990; Kaang et al., 1993).

2.7.5 Calcium/calmodulin-dependent protein kinase IV (CaMKIV)

CaMKIV, a transcriptional activator, is present in the nuclei and cytosol of neurons of different brain areas including the hippocampus (Jensen et al., 1991b; Jensen et al., 1991a). Upon MHFS, the levels of CaMKIV increase and as a result phosphorylates and activates CREB (Bito et al., 1996; Tokuda et al., 1997) (fig. 3). Mice with mutation in CaMKIV show impairment in L-LTP, long-term memory and CREB phosphorylation in the

hippocampus (Ho et al., 2000; Kang et al., 2001). However, these mutant mice have normal E-LTP and short-term memory.

2.8. Sleep and memory

Sleep is a natural physiological process hallmarked by the cyclic occurrence of two main stages: non-rapid-eye movement (non-REM) sleep followed by a much shorter period of rapid-eye movement (REM) sleep (Stones, 1977; Smith, 1995). As the night proceeds the length of NREM decreases, whereas the length of REM sleep increases. Non-REM sleep includes slow-wave sleep (SWS; stages 3 and 4) and lighter sleep (stages 1 and 2) (fig. 4A). SWS is characterized by different field potential oscillation patterns (slow oscillation, spindle, and sharp wave- ripple) (fig. 4B). On the other hand, REM sleep is characterized by muscle paralysis as well as by ponto-geniculo-occiptal waves and theta waves (fig. 4B) (Diekelmann and Born, 2010). Much of the literature supports a long-term integrative or consolidative role for different phases of sleep in recently acquired memory (Stickgold, 2005; Walker and Stickgold, 2006).

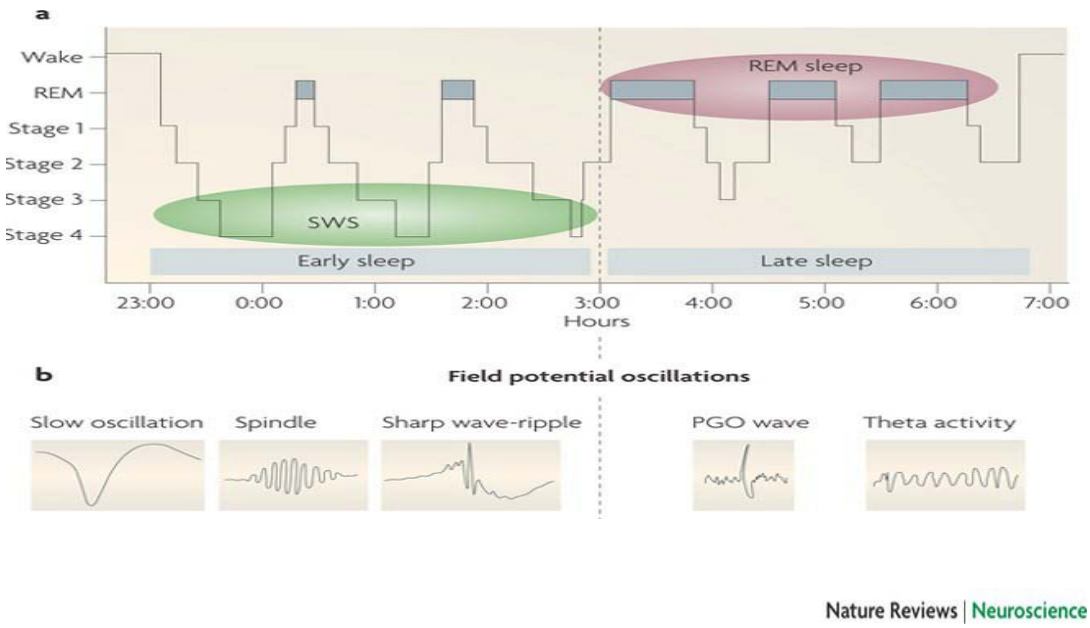


Figure 4: Stages of sleep and their neurphysiological features. (a) Sleep is hallmarked by cyclic occurrence of two main stages: NREM and REM. (b) Patterns of field potential rhythms in NREM and REM phases of sleep (Diekelmann and Born, 2010).

Two main hypotheses have been introduced to elucidate the mechanism behind the role of sleep in memory consolidation, the synaptic homeostasis and active system consolidation hypothesis (Tononi and Cirelli, 2006; Marshall and Born, 2007). During the awake state, declarative memory traces are encoded into a fast-learning temporary store represented by the hippocampus and a slow-learning permanent store corresponding to the neocortex. As SWS progresses, slow field oscillations synchronize to evoke widespread depolarization allowing reactivation and gradual redistribution of encoded memories from the temporary store to the long-term store (fig. 5)(Diekelmann

and Born, 2010 ; McClelland et al., 1995). This active system consolidation overlaps with the synaptic homeostasis hypothesis, which suggests that weaker synaptic connections are relatively eliminated. The depotentiation of weak synapses allows stronger connections to be preserved, thereby, preventing the saturation of synapses during reactivation (Tononi and Cirelli, 2006). After the integration of memories into a pre-existing long-term memories system, the REM phase disentangles the brain by disconnecting neural systems between the temporary (hippocampus) and long-term memory (neocortex) depots. This disentanglement subsequently allows for regional synaptic consolidation processes to take place (fig. 5), which further improve the memory representations that previously went through consolidation (Diekelmann and Born, 2010).

Accumulating reports show that sleep contributes significantly to the process of memory and neuronal plasticity (Samkoff and Jacques, 1991; Blissitt, 2001; Peigneux et al., 2001; McDermott et al., 2003). It is also known that adequate sleep is essential for fostering connections among neuronal networks for memory consolidation in the hippocampus (Kim et al., 2005; McDermott et al., 2006). In fact, hippocampal activity increases during sleep after a learning task (Gais et al., 2007) and sleep has been shown to increase hippocampus dependent memory (Cai et al., 2009).

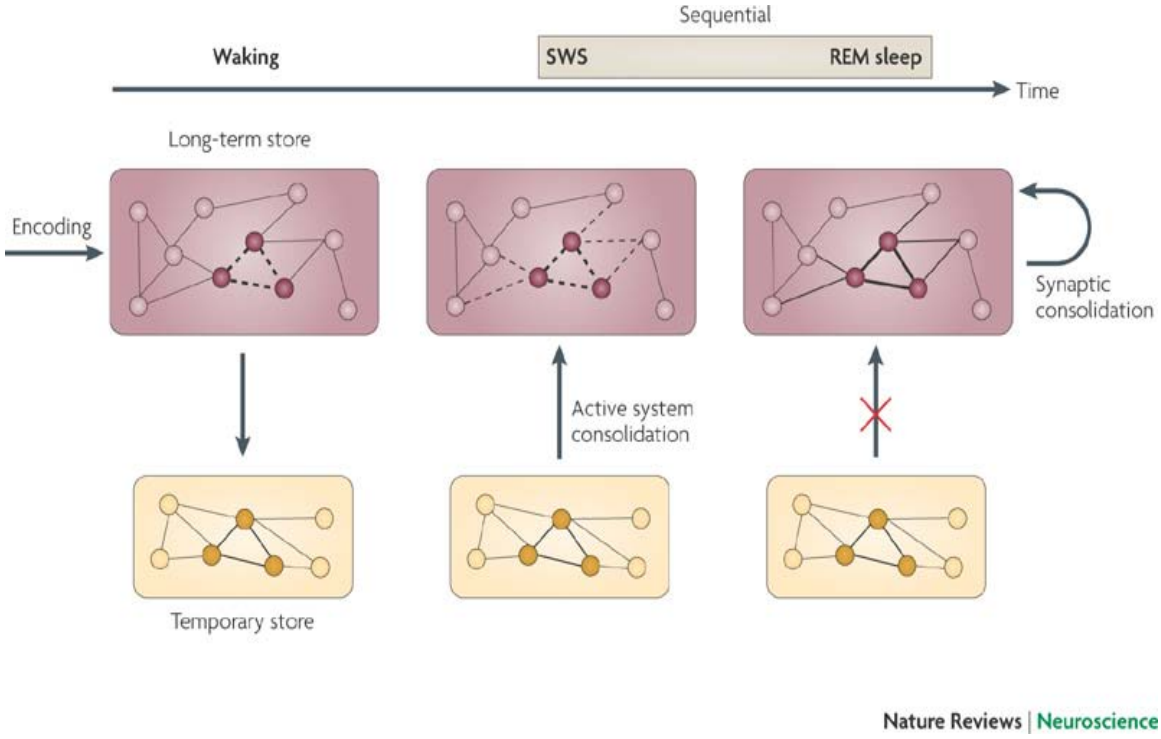


Figure 5: Sequential contribution of NREM and REM sleep to memory consolidation in short-term memory and long-term memory stores. SWS mediates the reactivation and redistribution processes of encoded memories from the temporary store to permanent store. REM phase disentangles the brain by dissociating neural systems, which allows for regional synaptic consolidation processes (Diekelmann and Born, 2010).

2.8.1. Sleep Deprivation Paradigms

The impact of sleep on cognitive function can be assessed by employing sleep deprivation as a model. Sleep deprivation is regarded as an extreme case of sleep loss in which a subject is awake for an extended period of time. Many useful approaches have been utilized to evaluate the role of sleep loss on cognitive functions, each with relative

advantages and disadvantages. In the following, I will discuss the commonly used methods for sleep deprivation.

2.8.1.1. Gentle handling paradigm

The gentle handling paradigm, also known as “hand deprivation” or “novel objects exposure”, includes introducing a tactile, olfactory or visual stimulus when the animals enter a particular prohibited sleep phase (REM or non-REM) as noted by electroencephalographic (EEG) recordings or visual observation (Tobler et al., 1990; Tobler and Scherschlicht, 1990; Franken et al., 1993; Ocampo-Garces et al., 2000; Vyazovskiy et al., 2002a; Modirrousta et al., 2005). Drawbacks of the gentle handling method involve the requirement of dedicated and continuous supervision by the examiners in addition to the issue that animals can become accustomed to the novel stimuli, thereby significantly limiting the duration of sleep loss. In addition, introduction of stimuli to an environment may not only keep animals awake, but it also becomes a contaminant by enriching the animal’s environment and perhaps confounding further behavioral tasks (Kopp et al., 2007).

2.8.1.2. Forced locomotion paradigm

Forced locomotion paradigms, such as the rotating disc over water or treadmill method, have been shown to reduce total sleep (REM and NREM equally) to around one

tenth of baseline after 24 hr of total sleep deprivation (Stefurak et al., 1977) Borbély et al., 1979). The forced treadmill paradigm applies forced locomotion to sleep-deprive the subjects (Levitt, 1966; Guzman-Marin et al., 2003). Additionally, the animals frequently lose muscle tone after sleep deprivation implying fatigue as a probable confounder (Webb and Agnew, 1962).

The rotating disc over water paradigm consists of a metal disc suspended over a shallow pool of water. Electroencephalographic (EEG) recordings are then utilized to identify when the sleep-deprived animal enters a prohibited sleep phase. Upon reaching the desired sleep stage, the disc is rotated (low speed of 1.33 rpm) and the animal must awaken to avoid falling into the water (Stefurak et al., 1977; Campbell et al., 2002). One obvious limitation is the fact that both the rotating disc and treadmill techniques depend on forced activity, which can lead to confounder stress and decreased LTP (Stefurak et al., 1977; Campbell et al., 2002; Guzman-Marin et al., 2003; O'Callaghan et al., 2007).

2.8.1.3. Modified multiple platform paradigm

The modified multiple platform technique, also known as the “water tank” or “columns-in-water” or “inverted flowerpot” paradigm, was designed for REM sleep deprivation. Although this method is effective in suppressing about 95% of REM sleep, it can interfere with NREM sleep as well (Grahnstedt and Ursin, 1985; Machado et al.,

2004). The single inverted flowerpot method was proposed by Jouvet in 1964 (Suchecki et al., 1998). This technique consists of a single platform or upside down flower pot immersed in a water tank. The animal is placed on top of a small platform, which is about 2 cm above the pool. Accordingly, sleep deprivation is achieved when the animal, upon entering REM sleep and losing muscle tone, comes in contact with the water and awakens (Van Hulzen and Coenen, 1980, 1981; Suchecki and Tufik, 2000; Rechtschaffen and Bergmann, 2002). However, the fact that the animal is not able to move inside the platform introduces isolation stress as a confounding factor. Thereafter, the multiple platforms method was developed to alleviate forced immobility associated with the single flowerpot method, thereby allowing freedom of movement. After that, the multiple platform technique was expanded into the less intrusive modified multiple platform method, which allows multiple animals from the same cage to undergo sleep deprivation together. The new adaptation addresses psychosocial, immobilization and isolation stress as confounders often observed in the previous flowerpot paradigms (Suchecki and Tufik, 2000; Machado et al., 2004).

2.8.1.4. Head-lifting

The head-lifting method is believed to be more specific for REM sleep deprivation. The researcher utilizes polygraphic recordings to recognize the onset of REM sleep in an animal housed in a different room. In this method, the researcher

pushes a mechanical lever, which is connected to the rat through a series of pulleys and a flexible wire within 2-3 seconds of REM onset. As a result, the animal's head is gently lifted by 2 inches in order to awaken the animal (Datta et al., 2004). This method is useful in eliminating REM sleep episodes within a 3-5 second window, making it REM sleep specific, but it results in a laborious and time consuming method. Additionally, this method is neither applicable for long durations of time nor it is useful for multiple animals.

2.8.1.5. Enlarged environment

The enlarged environment paradigm method has been developed to accomplish total sleep deprivation, lasting about four hrs. In this method, sleep loss is carried out by stimulating the animal's natural tendency to explore novel surroundings by providing the animal with an enlarged environment. Furthermore, this method has been reported to be a stress-free sleep deprivation paradigm since the levels of corticosterone were unaltered after sleep loss using this paradigm (Kopp et al., 2006). However, the possible confounding influence of the enlarged environment method on the learning process of behavioral tasks can not be excluded (Kopp et al., 2007).

2.8.2. Sleep deprivation, memory, and synaptic plasticity

There is a large body of evidence showing a strong correlation between sleep deprivation and memory impairment in humans and animals (Polzella, 1975a; McDermott et al., 2003; Guan et al., 2004; Ferrara et al., 2008). Sleep loss impairs hippocampus-dependent learning and memory (Youngblood et al., 1997; Smith et al., 1998; McDermott et al., 2003; Guan et al., 2004; Tartar et al., 2006). Although sleep deprivation suppresses LTP in hippocampal CA1 (McDermott et al., 2003; Kim et al., 2005; Kopp et al., 2006; Tartar et al., 2006) and DG areas (Marks and Wayner, 2005; Ishikawa et al., 2006), LTD that is induced by paired-pulse facilitation is not affected after 24 hr of sleep loss (Tartar et al., 2006).

The harmful effect of sleep deprivation on spatial memory and hippocampal LTP may be related to the disruption of molecular signaling cascades. This is supported by the findings that lack of sleep alters the gene expression of CaMKII and CREB (Guzman-Marin et al., 2006), which are important regulators of short-term memory and long-term memory, respectively. Additionally, sleep deprivation reduces protein levels of BDNF and the phosphorylated form of the extracellular signal-regulated kinase 2 (P-ERK2) (Guan et al., 2004; Guzman-Marin et al., 2006). Emerging reports show that sleep loss lowers excitatory postsynaptic currents and decreases the GluN1 subunit of NMDA receptors of the CA1 area (Chen et al., 2006; Ravassard et al., 2009).

2.9. Caffeine and memory

Caffeine, a commonly used central nervous system stimulant (Nicholson and Stone, 1980; Nehlig et al., 1992; Ferre, 2008), is present in several beverages and food. Caffeine can prevent cognitive impairments and neurodegeneration in different models of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases (Gevaerd et al., 2001; Dall'Igna et al., 2004; Arendash et al., 2006; Dall'Igna et al., 2007). Interestingly, epidemiological studies also support that caffeine intake is inversely correlated with the incidence of Alzheimer's or Parkinson's disease (Ascherio et al., 2001; Maia and de Mendonca, 2002; Ritchie et al., 2007). The beneficial effects of caffeine administration on neurodegenerative disorders may be related its non-selective antagonism of adenosine receptors (Nehlig et al., 1992; Takahashi et al., 2008; Pires et al., 2009).

Caffeine exhibits affinities for different types of receptors present in the synaptic membranes and cytoplasmic calcium stores. On the synaptic membrane level, caffeine blocks adenosine receptors (Kerr et al., 1991; Fredholm et al., 1999), mainly adenosine A_1 (A_1) and adenosine A_{2a} (A_{2a}) receptors (fig. 6A). On the other hand, caffeine activates ryanodine receptors, which causes an increase in calcium-induced calcium release from cytoplasmic calcium stores (fig. 6B) (McPherson et al., 1991), leading to an increase in calcium-dependent signaling pathways. Additionally, caffeine inhibits phosphodiestrase

(PDEIV) enzyme (Smellie et al., 1979); and, as a result, intracellular cyclic adenosine mono-phosphate (cAMP) is increased (fig. 6C), enabling caffeine to enhance the cAMP signaling cascades.

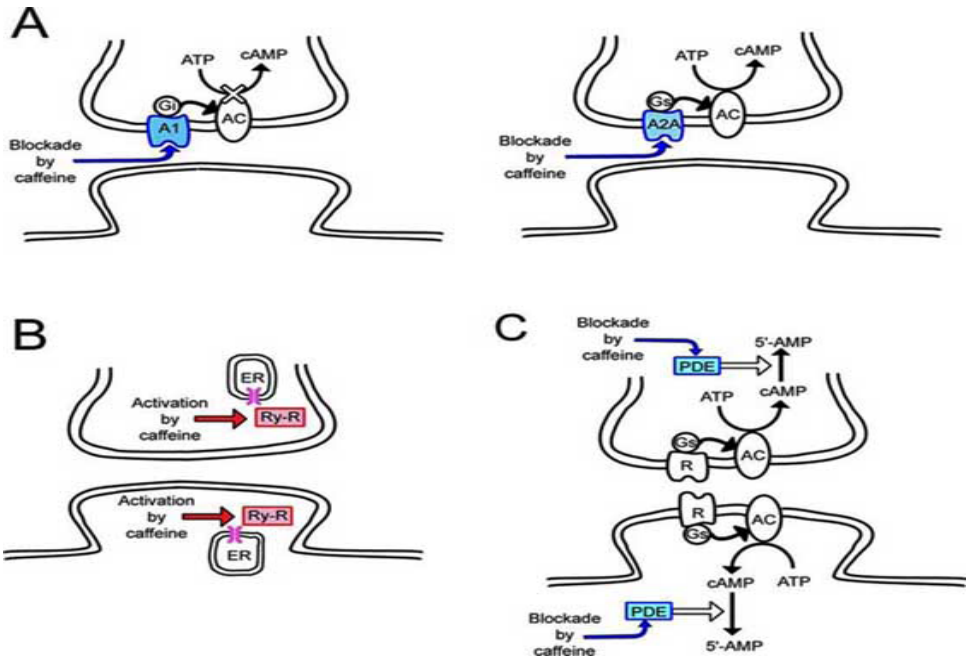


Figure 6: Mechanisms of action of caffeine. Caffeine can act through several mechanisms including (A) antagonism of adenosine receptors, (B) increasing calcium induced-calcium release, and (C) phosphodiesterase enzyme inhibition (Yoshimura, 2005).

2.9.1. Caffeine, memory and synaptic plasticity

Evidence has shown that caffeine is implicated in the modulation of learning and memory tasks. The impact of caffeine on cognitive function is still a matter of debate.

Whereas much evidence demonstrates a beneficial effect of caffeine in both human (Riedel et al., 1995; Riedel and Jolles, 1996) and animal (Roussinov and Yonkov, 1976; Cestari and Castellano, 1996; Howell et al., 1997) models of learning and memory, other studies show that caffeine does not alter normal memory (Loke, 1988; Furusawa, 1991; Hudzik and Wenger, 1993), or even harms it (Terry and Phifer, 1986; Sansone et al., 1994; Fisher and Guillet, 1997).

The literature supports the positive influence of caffeine on synaptic transmission efficiency. In fact, caffeine treatment has been shown to modify synaptic activities by inducing LTP in the synapses of area CA1 pyramidal neurons *in vitro* (Lu et al., 1999a; Martin and Buno, 2003). Consistent with this observation, caffeine can mediate structural changes in cultured-hippocampal neurons by enhancing the size of dendritic spines (Korkotian and Segal, 1998, 1999). On the molecular level, caffeine treatment increases the levels of BDNF in the hippocampus of caffeine-treated mice, while the levels of P-CREB remain unaltered (Costa et al., 2008b).

2.9.2. Caffeine and adenosine receptors

The mechanism that underlies the action of relevant concentration of caffeine for human consumption is probably blockade of adenosine A_1 and A_{2a} receptors (Fredholm, 1995). Adenosine A_1 and A_{2a} receptors are coupled with inhibitory and stimulatory G-proteins, respectively. As a result, activation of A_1 receptors inhibits

adenylyl cyclase (AC), whereas A_{2a} activation stimulates AC (Dixon et al., 1996; Dunwiddie and Masino, 2001). In neurons, A_1 and A_{2a} receptors are expressed at presynaptic terminals as well as at postsynaptic membranes (Fredholm et al., 1999). Adenosine A_1 receptors are abundant in the hippocampus and neocortex, where it is linked to glutamatergic system (Dixon et al., 1996; Dunwiddie and Masino, 2001). Conversely, A_{2a} receptors are highly expressed in the striatum and, therefore, linked with the dopaminergic system (Fink et al., 1992; Ferre et al., 1997).

It is well established that in the hippocampus, endogenous adenosine interferes with synaptic plasticity through the highly expressed adenosine A_1 receptors. Adenosine produces an inhibitory effect on LTP in the CA1 area in the rat hippocampal slices (de Mendonca and Ribeiro, 1994). Adenosine also disrupts the underling processes of learning and memory at the synaptic level. At the pre-synaptic neuron, adenosine inhibits the release of excitatory neurotransmitter (glutamate) from nerve terminals; and, at the post-synaptic neurons, adenosine stabilizes the magnesium ion on NMDA receptors and, accordingly, prevents NMDA receptor-mediated excitation (de Mendonca et al., 1995; Dunwiddie and Masino, 2001).

3. MATERIALS AND METHODS

3.1. Animal and housing conditions

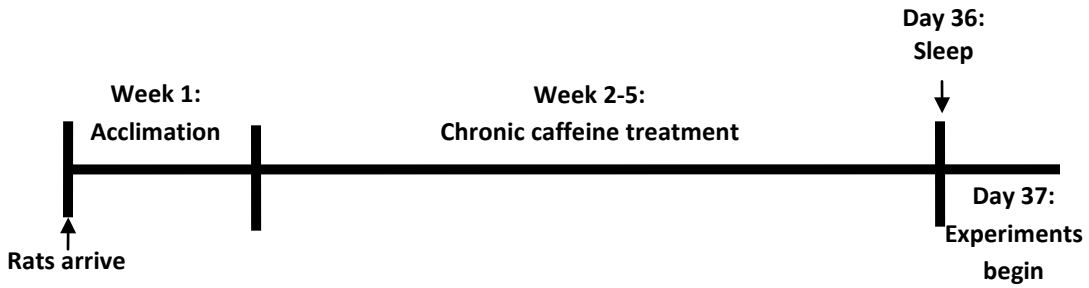
All animal experiments were carried out in accordance with the National Research council's Guide for The Care and Use of Laboratory Animals and on approval of University of Houston Institutional Animal Care and Use Committee. We used adult male Wistar rats (Charles River Laboratories, Wilmington, MA), weighing 175-200g at the beginning of the study, and housed 5 rats to a plexiglas cage in a climate-controlled room (25 °C) on a 12/12-hr light/dark schedule (lights on at 7 am) with ad libitum access to standard rodent chow and water. After arrival at the animal care facility, all rats were allowed to acclimate for one week before starting the experiments. Four experimental groups were designated; control, sleep deprivation, caffeine and caffeine/sleep deprivation. All experiments were carried out between 8 AM and 5 PM.

3.2. Treatments

3.2.1. Caffeine treatment

Rats in the caffeine and caffeine/sleep deprivation groups were allowed to drink only caffeinated (Sigma Aldrich, Saint Louis, MO) tap water from water bottles (0.3 g/l) ad libitum for four weeks (Rigoulot et al., 2003) (scheme 1). The concentration of caffeine used in the present study approximately equals to the average daily human

consumption of caffeine (Han et al., 2009). On average, the amount of water consumed by the caffeinated rats and non-caffeinated rats was similar. The estimated amount of caffeine that each rat consumed daily was 16-20 mg. During sleep deprivation of the caffeine/sleep deprivation group, we added the same concentration of caffeine in the aquarium water in case the rats drink from aquarium water rather than water bottles.



Scheme 1: Treatment of rats during the course of the experiment.

3.2.2. Sleep deprivation

Sleep consists of two main stages non-rapid eye movement (NREM) and REM sleep. The two stages occur alternately across the night in 4-5 cycles (Blissitt, 2001). Wister rats in the sleep deprivation and caffeine/sleep deprivation groups were sleep-deprived for a 24 hr period (scheme 1) using the columns-in-water method; an adaptation for the modified multiple platform model. Loss of muscle tone during REM sleep phase caused the rats to fall into the water and awaken. This method has been reported to interfere with both NREM and REM sleep, but it mainly eliminates REM

sleep (Grahnstedt and Ursin, 1985). Five rats from the same cage were placed in a large aquarium at room temperature (24 °C ± 1 °C). The aquarium contained twenty columns (diameter: 5 cm, with platforms 2 cm above the water level), spaced 7 cm apart (edge to edge), arranged in 2 rows such that rats could move freely from one platform to another. During the sleep deprivation period, rats had free access to water bottles and food pellets baskets hanging from the aquarium cover (McDermott et al., 2006). Additionally, to test the effect of possible stresses of the aquarium environment, we kept the rats of the wide platform group in the aquarium. For this purpose, we used wide platforms (12 cm in diameter) in order to allow the rats in the wide platform group to sleep without falling into the water. This group was used in the behavioral study only, where they were subjected to learning, short-term memory, and long-term memory tests in the RAWM.

3.3. Behavioral Experiments: Radial Arm Water Maze procedure

All five experimental groups (control, sleep deprivation, wide platform, caffeine and caffeine/ sleep deprivation) were tested for spatial learning and memory performance in the radial arm water maze. The radial arm water maze consisted of a black circular tub filled with clear water (24 °C ± 1 °C) with six V-shaped stainless steel structures arranged to form an open central area and six swim paths (Devan et al., 1996; Diamond et al., 1999; Aleisa et al., 2006d; Alzoubi et al., 2009a). The

experiments were carried out in a small dimly-lit room with various large visual signs on the walls. A rat had to find a submerged platform (2 cm below the water level) at the end of the "goal" arm, probably aided by the fixed visually cues on the walls. Starting from an arm other than the goal arm, each rat was allowed to locate the hidden platform within one min. An error was recorded each time the rat entered into an arm other than the goal arm. Once the rat located the platform, it was allowed to stay on it for 15 sec before the beginning of the next trial. When a rat failed to find the platform within the one min period allowed, the experimenter would guide it toward the platform.

In this study, we used a one-day radial arm water maze protocol to test learning and short-term memory and long-term memory as reported (Alzoubi et al., 2009b). To test learning and short-term memory, the rats in sleep deprivation and caffeine/sleep deprivation groups were sleep-deprived for 24 hrs. Then in RAWM, rats were initially subjected to six learning trials (trials # 1-6) followed by a five min rest period and then another six learning trials (trials # 7-12). The animals were tested for short-term memory 30 min after the end of 12^{th} trial. To test the long term memory, we used a slightly different protocol, the post-learning sleep deprivation protocol, where all the five groups went through two blocks of six learning trials. After the end of the 12^{th} trail, we sleep-deprived the rats in the sleep deprivation and

caffeine/sleep deprivation groups for 24 hrs. Immediately after that, we subjected the rats to the long-term memory test.

3.4. Electrophysiological Experiments

Electrophysiological recording of population spike (pSpike) from anesthetized animals was performed as reported (Gerges et al., 2001; Gerges et al., 2003a; Gerges and Alkadhi, 2004; Alzoubi et al., 2005a; Aleisa et al., 2006b). A new set of four groups of rats (control, sleep deprivation, caffeine and caffeine/sleep deprivation) were anesthetized with urethane (1.2 g/kg ip) at the end of treatments. Rat was positioned in a stereotaxic frame (nose bar at 0.0) for standard extracellular electrophysiological recording from hippocampal CA1 and DG areas. Then the skull was exposed and holes were drilled. A heat pad or heating lamp was used to maintain normal body temperature of the rat. Additionally, the rat was grounded through a subcutaneous wire in order to eliminate electrical noise.

Positioning of the stimulating and recording electrode in the CA1 area

For LTP induction in the CA1 area, a concentric bipolar stimulating electrode (twisted tephlon-coated stainless steel wires) was placed through a predrilled hole, in area CA3 at an angle of 5 degrees toward the midline (anterior posterior [AP], -3; lateral [L], 3.5; dorsal [D] 2.8). A glass recording microelectrode (tip resistance; 1-5 mΩ) filled

with 1% Fast Green dye in 2 M NaCl, was inserted through another predrilled hole in stratum pyramidale of CA1 area of the right hippocampus (AP, -3; L, 1,8; D, 2.0). The left CA3 region was stimulated and responses were recorded from the right CA1 region (fig. 7).

Positioning of the stimulating and recording electrode in the DG area

For LTP induction in the DG area, a bipolar stimulating electrode was placed in the right angular bundle (AP, -8; L, 4.4; D, 3). A glass recording microelectrode was placed in the granule cells of DG area of the right hippocampus (AP, -3; L, 1.8; D, 3.0). The right angular bundle region was stimulated and responses were recorded from the right DG region (fig. 7).

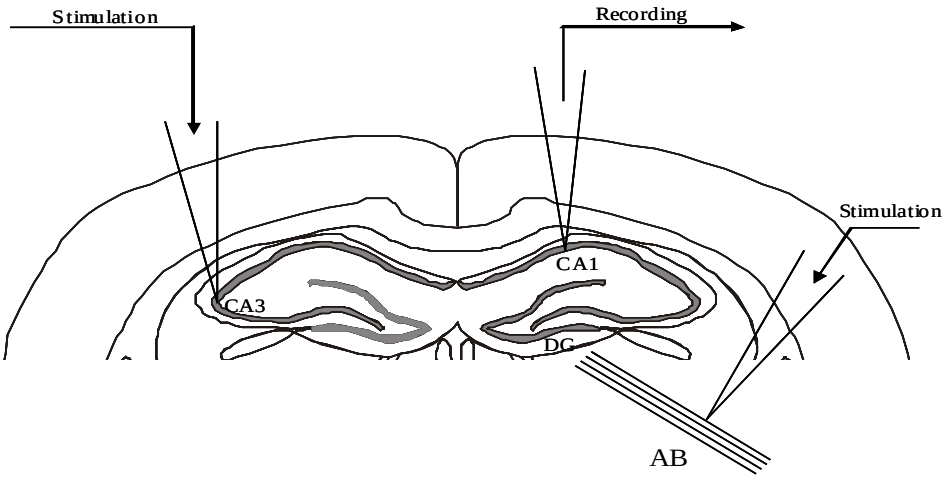
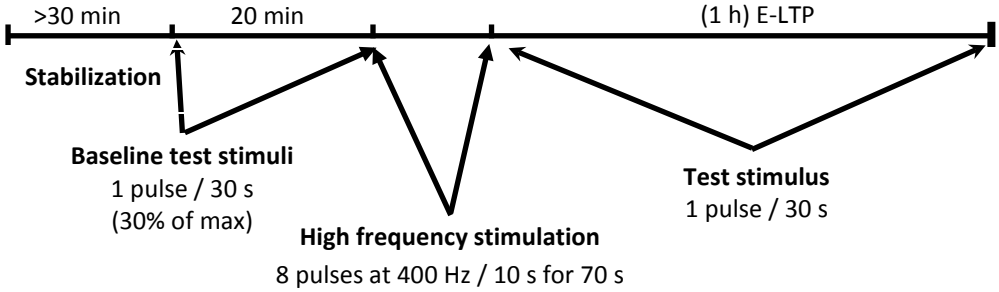


Figure 7: Positioning of the stimulating and recording electrodes. The left CA3 region was stimulated to record from right CA1 region. The recording electrode in the right DG area recorded responses evoked by stimulation of the angular bundle (AB).

The position of the recording electrode was adjusted to evoke population spikes (pSpike) by infrequent stimulation in the pyramidal cell layer of the CA1 area or in the granule cell layer of the DG area. After a stabilization of the response of about 30 min, input-output (I/O) curves were generated by gradually increasing the stimulus intensities (input) and recording pSpikes generated (output). Thereafter, a stimulus intensity of approximately 30 % of the maximum response was chosen to evoke test responses. A baseline recording was achieved by giving a test stimulus, adjusted to evoke approximately 30% of the maximal response, every 30 sec for a period of 20 min. Similar test stimuli were applied for one hr after HFS or five hrs after MHFS (Alzoubi et al., 2005b; Aleisa et al., 2006a; Aleisa et al., 2006d; Alzoubi et al., 2007a)

3.4.1. E-LTP induction protocol

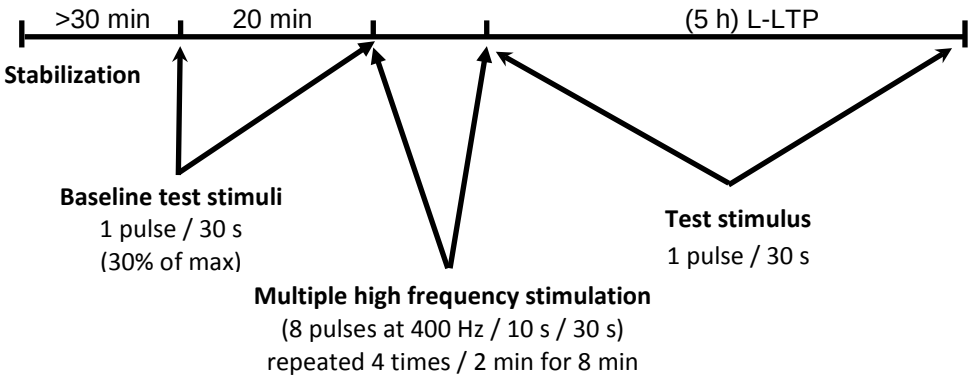
Early phase-LTP was induced by applying HFS to the Schaffer collaterals/commissural pathway or to the perforant path. The HFS consisted of a train of 8 pulses (400 Hz) applied every 10 sec for a period of 70 sec (scheme 2).



Scheme 2: Stimulation protocol for induction of E-LTP.

3.4.2. L-LTP induction protocol

In a separate group of rats, MHFS trains were applied to Schaffer collaterals/commissural pathway or the perforant path to evoke L-LTP. MHFS consisted of 4 trains with 2.5 min interval between the trains, and each train consisted of eight pulses (400 Hz) given every 10 seconds for a period of 30 seconds (scheme 3).



Scheme 3: Stimulation protocol for induction of L-LTP.

Evoked pSpikes were recorded from the CA1 and DG regions of the right hippocampus for 60 min after HFS, or five hrs after MHFS, and amplified (Axoclamp 2A amplifier, Axon Instruments, Inc., Foster City, CA, USA). The slope of fEPSP and amplitude of pSpike were measured as described in figure 8. Briefly, the slope of fEPSP (mV/ms) was measured from upstroke between the two lines as shown in figure 8, whereas the amplitude of pSpike (mV) was measured by calculating the voltage

difference from the mid-point of the two peaks (the dashed lines) to the lowest point of the spike (see fig. 8). Computer-based stimulation and recording were achieved by using pCLAMP 10.2 software and DigiData 1440A (Axon Instruments, Inc.). All recorded responses were normalized to the average value of the baseline. The amplitudes of the pSpike and values of the slope of the fEPSP at each point in graphs were averaged from 10 consecutive traces.

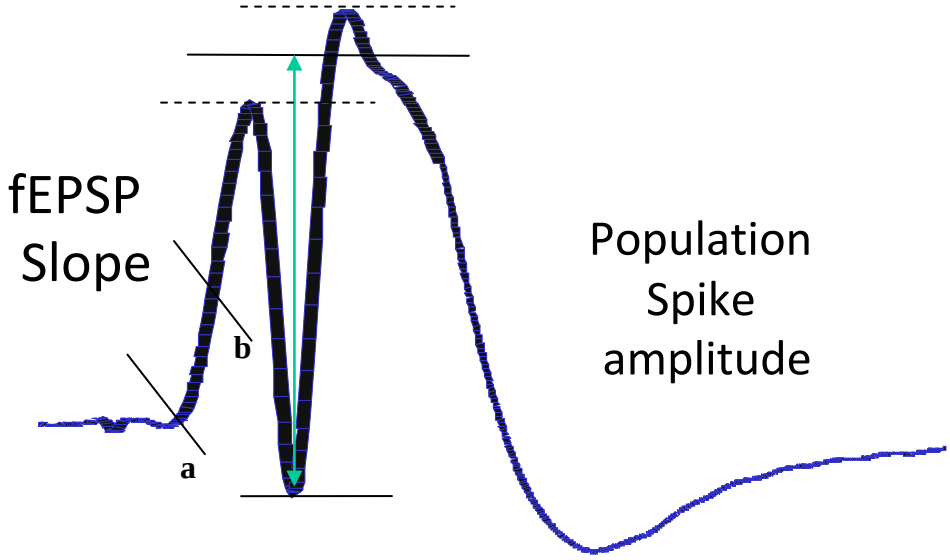


Figure 8: The method of measurement of fEPSP slope and pSpike amplitude. The trace reflects a typical induced population spike (pSpike) recorded from the DG area upon stimulation of the angular bundle. The amplitude of the pSpike was measured from the mid-point between the dashed lines to the lowest point of the spike. Slope of fEPSP is measured from upstroke between the two lines (a and b) as shown (mV/ms).

The slope of fEPSP provided a reliable measure of the strength of synaptic activity that resulted from presynaptic stimulation, whereas the amplitude of pSpike

provided a measure of the number of postsynaptic neurons reaching threshold from this synaptic input. We considered the increase in the slope of fEPSP, which lasts at least one hr after HFS a measure of E-LTP. The increase in the slope of fEPSP measured four hrs after MHFS was considered as a measure of L-LTP.

3.5. Molecular Experiments

3.5.1. Brain dissection and processing

For the basal levels of the signaling molecules, the rats were sacrificed after the end of treatment and the right hippocampi were removed to dissect out the right CA1 or DG areas as reported (Gerges et al., 2003b; Aleisa et al., 2006c; Alzoubi et al., 2006; Alzoubi and Alkadhi, 2007). To determine the stimulated levels of signaling molecules in the CA1 or DG area, one hr after induction of E-LTP or five hrs after induction of L-LTP, the right hippocampi were removed, and immediately positioned on filter paper soaked in 0.2 M sucrose (to avoid brain sticking to filter paper) over a covered petri dish containing dry ice. The two tips of the hippocampus were trimmed. Then, the hippocampus was cut into three parts: septal (dorsal), temporal (ventral), and middle. The septal and temporal parts of the hippocampus were placed in a vertical position to dissect CA1 or DG area under a light microscope. We considered the temporal part as un-stimulated internal control since most of the stimuli went into the septal portion of the same right hippocampus (fig. 9) (Tamamaki et al., 1988; Papatheodoropoulos and

Kostopoulos, 2000). Previous reports from this lab have shown that levels of signaling molecules in un-stimulated control rat are not significantly different in the septal and temporal sides of the same CA1 area (Gerges et al., 2004a). Therefore, the levels of signaling molecules in septal side of area CA1 were expressed as percent of those levels in the temporal side of area CA. The samples were placed into prelabeled microcentrifuge tubes and then stored at -80 C until homogenization and processing.

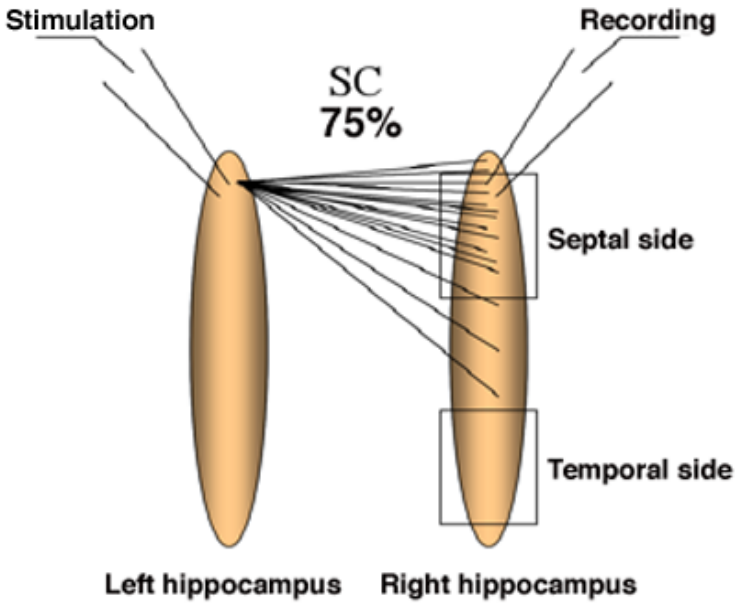


Figure 9: Septo-temporal structure of the hippocampus during stimulation. Stimulation of the septal side of the left Cornu Ammonis (CA)-3 area of the hippocampus induces responses recorded in the septal side of the right CA1 area of the hippocampus. It has been reported that each CA3 neuron synapses to as much as 75% of the septal part of ipsilateral and contralateral CA1 fields, whereas the temporal part receives negligible inputs (Tamamaki et al., 1988). Thus, we used the temporal part of the right CA1 region of the hippocampus as unstimulated "internal" control.

3.5.2. Homogenization and preparation of samples

Preparation of tissues for immunoblot analysis was carried out as reported (Tran et al., 2010 ; Srivareerat et al., 2008). protein extracts from hippocampal tissues were homogenized separately in 200 µl of lysis buffer cocktail containing protease and phosphatase inhibitors (Tris-HCl pH 7.4, 50 mM; NaCl, 150 mM; PMSF, 1 mM; NaF, 50 mM; EDTA, 1 mM; EGTA, 1 mM; Na4P2O7 15 mM; B-glycerophosphate; 40 mM; nonidet P-40, 1%; SDS, 0.1%) by using polytron homogenizer PRO250 (PRO Scientific, Oxford, CT) at a medium speed for five sec, repeated three times. The homogenates were then sonicated three times for five sec each using an ultrasonicator (Vibra cell, Sonics & Materials Inc., Newtown, CT). Total protein was estimated by micro bicinchoninic acid (BCA) assay (Pierce Chemical Rockford, IL), and then stored at -80 °C until use.

3.5.3. Immunoblotting analysis

Immunoblotting technique was carried out through six steps. The first step is the sample preparation, where the tissue homogenates were removed from the freezer, left to thaw on ice, diluted with 4X E-PAGETM loading buffer and 10 X NuPAGE® reducing agent (Invitrogen, Carlsbad, CA) and then heated at 70° C for 10 min. In the second step, the gel electrophoresis, the same quantity of protein (10 µg) from each homogenate was loaded onto an 8%, 48-well protein electrophoresis gel (EPAGE 48, Invitrogen) and resolved by E-PAGE high throughput (HTP) protein electrophoresis system (E-BASE,

Invitrogen). For the third step, transfer, the proteins on the pre-cast gel were transferred to polyvinylidine fluoride (PVDF) membrane through the buffer-less, dry blotting system (iBlot, Invitrogen). The membrane was activated by presoaking in methanol (95%; approximately for one min). In the fourth step, the blocking, the PVDF membranes were soaked in 5% nonfat dried milk or albumin, from bovine serum (BSA) in TBS/Tween for 60 min to block any non-specific binding. The fifth step included addition of the antibodies in which blots were first incubated with primary antibody (table1). The incubation period could be either one hr at room temperature or overnight at 4 °C. To remove the excess of primary antibody, the membranes were washed three times with TBS/Tween. After that, the membranes were incubated with horseradish peroxidase enzyme-conjugated secondary antibody (table 1) for one hr at room temperature. The membranes were then washed with TBS/Tween before the final, detection step. The blots were developed using chemiluminescence reagent (ECL; Alpha Diagnostic, San Antonio, TX), and captured visually in the Alpha Innotech imaging system. The intensity of immunoreactive bands was then quantified by densitometry using Fluorchem FC8800 software.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as loading control, and the band intensities of all signaling molecules were expressed as a ratio to the GAPDH intensity.

In order to detect different proteins in the same blot, antibodies were removed from the blot using a stripping solution. In this process, blots were first washed with distilled water for five min, and then incubated with a stripping solution (0.2 M NaCl) for five min followed by a final five min wash with distilled water. After this, the stripped blots were ready for a new round of protein detection.

3.6. Statistical analysis

All of the groups were compared using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test where appropriate. P values < 0.05 were considered significant. All values were represented as mean ± standard error of the mean (SEM). Analyses were conducted using GraphPad Prism (4.0) (software, Inc. San Diego, CA).

	Primary antibody	Secondary antibody
P-CaMKII	Mouse monoclonal antibody anti-P-CaMKII (1:1000; Santa Cruz Biotechnology, Inc. CA)	Goat anti-mouse antibody, HRP (1:4000; Santa Cruz Biotechnology, Inc. CA)
Total CaMKII	Rabbit polyclonal antibody anti-CaMKII (1:1000; Santa Cruz Biotechnology, Inc. CA)	Anti-rabbit antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
Calcineurin	Rabbit polyclonal anti-calcineurin antibody (1:1000; Upstate Biotechnology, NY)	Anti-rabbit antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
BDNF	Mouse monoclonal anti-BDNF (1:500; Santa Cruz Biotechnology, Inc. CA)	Anti-mouse antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
P-CREB	Goat polyclonal antibody anti-P-CREB (1:1000; Santa Cruz Biotechnology, Inc. CA)	Anti-goat antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
Total CREB	Rabbit polyclonal antibody anti-CREB (1:1000; Santa Cruz Biotechnology, Inc. CA)	Anti-rabbit antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
CaMKIV	Rabbit polyclonal anti-CaMKIV (1:500; Cell Signaling Technology, MA)	Anti-rabbit antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)
GAPDH	Mouse monoclonal antibody against GAPDH (1:5000; Millipore, MA)	Goat anti-mouse antibody (1:4000; Santa Cruz Biotechnology, Inc. CA)

Table 1: Antibodies, dilutions and sources: Summary of the primary and secondary antibodies that were used for the different molecules.

4. RESULTS:

4.1. Behavioral results:

Frequent studies have shown that spatial learning and memory is a hippocampus-dependent task (Morris et al., 1982; Lopes da Silva et al., 1986; Sherry et al., 1992; Squire, 1993; He et al., 2001; Astur et al., 2002; Burgess et al., 2002) and that sleep deprivation impairs spatial learning and memory performance (Youngblood et al., 1997; Youngblood et al., 1999; Graves et al., 2003; Guan et al., 2004; Ruskin et al., 2004; Bjorness et al., 2005). In this section, we tested the effect of chronic low dose of caffeine on sleep deprivation-induced spatial learning and memory impairment using the radial arm water maze (RAWM) method. Additionally, the interference of possible stresses from the aquarium environment was evaluated by keeping the rats on the wide platform, which permitted them to sleep without falling into the water for a period of 24 hrs.

4.1.1. Chronic caffeine treatment averts spatial learning deficit associated with sleep deprivation

During the first 6 trials of the learning (acquisition) phase, rats in all groups learned to find the hidden platform at the same rate, as indicated by the decreasing number of errors in trials 1-6 (fig. 10A). During the second 6 learning trials, rats in the control, caffeine, wide platform and caffeine/sleep deprivation groups learned the

location of the hidden platform at equivalent rates. In contrast, the ability of the sleep deprivation group to find the hidden platform was significantly ($P < 0.05$) impaired in learning trials 7, 11 and 12 as indicated by the higher number of errors committed compared to the other experimental groups (fig. 10A). These results show that sleep deprivation impairs spatial learning and that chronic caffeine treatment prevents learning impairment and that performance of rats of the wide platform group is not significantly different from home-cage rats.

4.1.2. Chronic caffeine treatment averts spatial short-term memory deficit associated with sleep deprivation

In short-term memory test, administered 30 min after the end of the 12^{th} trial, the sleep deprivation group committed significantly ($F_{4,54} = 10.95$, $P < 0.0001$) more errors in finding the hidden platform in the RAWM than the control group (control: 0.83 ± 0.2; sleep deprivation: 3.75 ± 0.39) (fig. 10A, B) indicating marked impairment of short-term memory. Moreover, the average number of errors made by rats of the wide platform group (1.2 ± 0.47) in the short-term memory test was not different from those in the control group (fig. 10B) suggesting that short-term memory impairment associated with the columns-in-water model is probably due to sleep deprivation itself and not the aquarium environment. Chronic caffeine treatment prevented the increase in the number of errors in the sleep-deprived rats (1.1 ± 0.38) as indicated by the lack of

significant difference from the control group (fig. 10B). However, chronic caffeine treatment in normal rats had no significant effect on performance in the RAWM. These findings indicate that chronic caffeine treatment protects spatial short-term memory against the deleterious effects of sleep deprivation.

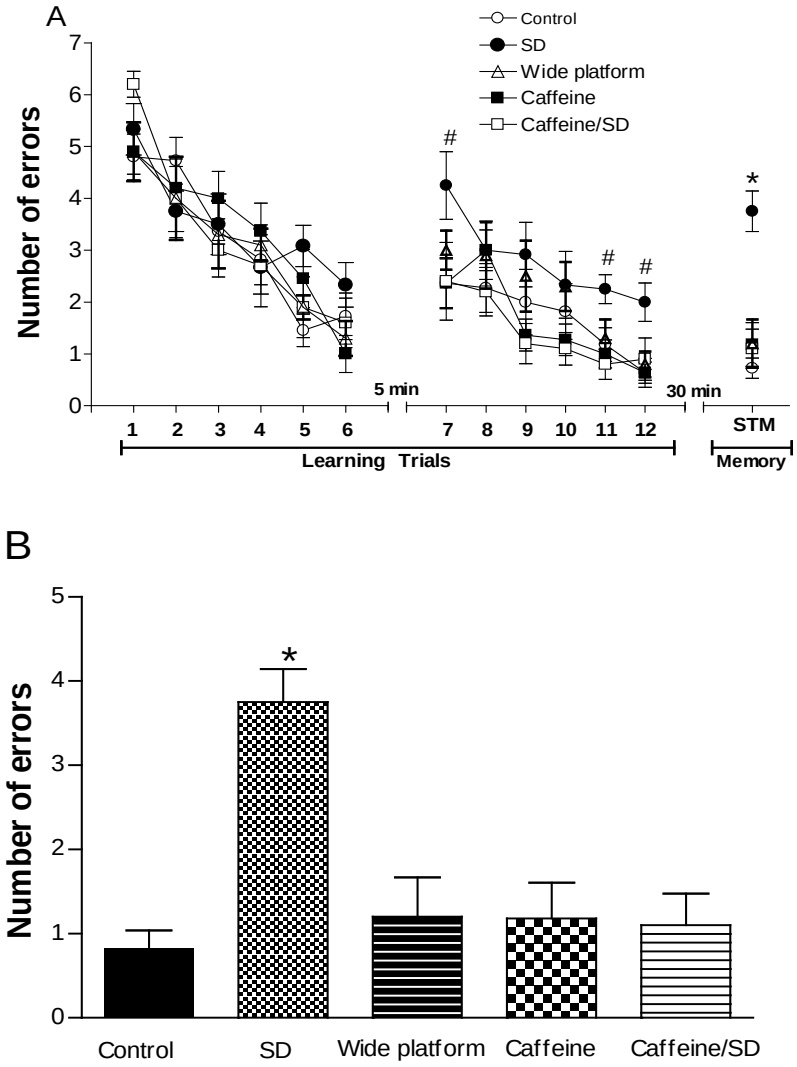


Figure 10: Effect of chronic caffeine treatment on sleep deprivation-impaired learning and short-term memory tests in the RAWM. (A) Sleep-deprived rats showed significant impairment of learning in trials number 7, 11, and 12 compared to rats in control, wide platform, caffeine, and caffeine/SD. Additionally, short-term memory was markedly impaired in sleep-deprived rats, but was normal in caffeine-treated rats. (B) The average number of errors made by each of the five groups in short-term memory test, 30 min after trial number 12. Chronic caffeine treatment before sleep deprivation prevented short-term memory impairment. Each point is the mean ± SEM of 10 to 12 rats. * ($P < 0.01$) And $^{\#}$ ($P < 0.05$) indicate significant difference from other groups.

4.1.3. Chronic caffeine treatment averts spatial long-term memory deficit associated with sleep deprivation

To test long term memory, we used a post-learning long-term memory protocol. In this protocol, all the five groups of Wistar rats designated as, control, sleep deprivation, wide platform, caffeine, and caffeine /sleep deprivation went through 6 consecutive learning trials then 5 minutes rest followed by another 6 consecutive learning trials without 24 hr sleep deprivation (fig. 11A). Then, immediately after the end of the acquisition phase, rats in the sleep deprivation, wide platform, and caffeine/sleep deprivation groups were placed in the columns-in-water model for a period of 24 hrs. During the acquisition phase, there were no differences in the behavioral performance among the five groups as indicated by the average number of errors scored to find the goal arm by each group (fig. 11A). Therefore, the sleep deprivation period between the learning phase and long-term memory test should reflect the effect of sleep deprivation on long-term memory.

Twenty-four hours after the end of trial number 12, the long-term memory performance was assessed by comparing the number of errors made by each treated group with the number of errors made by the control group. We found that the sleep deprivation group committed significantly ($F_{4,41} = 14.93$, $P < 0.0001$) more errors (4.6 ± 0.45) in finding the hidden platform in the RAWM than the control group (1.1 ± 0.27)

(fig. 11A, B) indicating marked impairment of long-term memory. Moreover, the average number of errors made by rats of the wide platform group (1.3 ± 0.49) in the long-term memory test was not different than that of the control group (fig. 11B), suggesting that the causative factor for long-term memory impairment is sleep deprivation rather than stresses that may be associated with the columns-in-water model. Chronic caffeine treatment for four weeks before sleep deprivation significantly reduced the number of errors (2.8 ± 0.34) made by the sleep deprivation group but did not fully restore it to that of the control group (fig. 11A, B). However, chronic caffeine treatment in normal rats had no significant effect on long-term memory performance in the RAWM. These findings indicate that chronic caffeine treatment prevents spatial long-term memory impairment in acutely sleep-deprived rats.

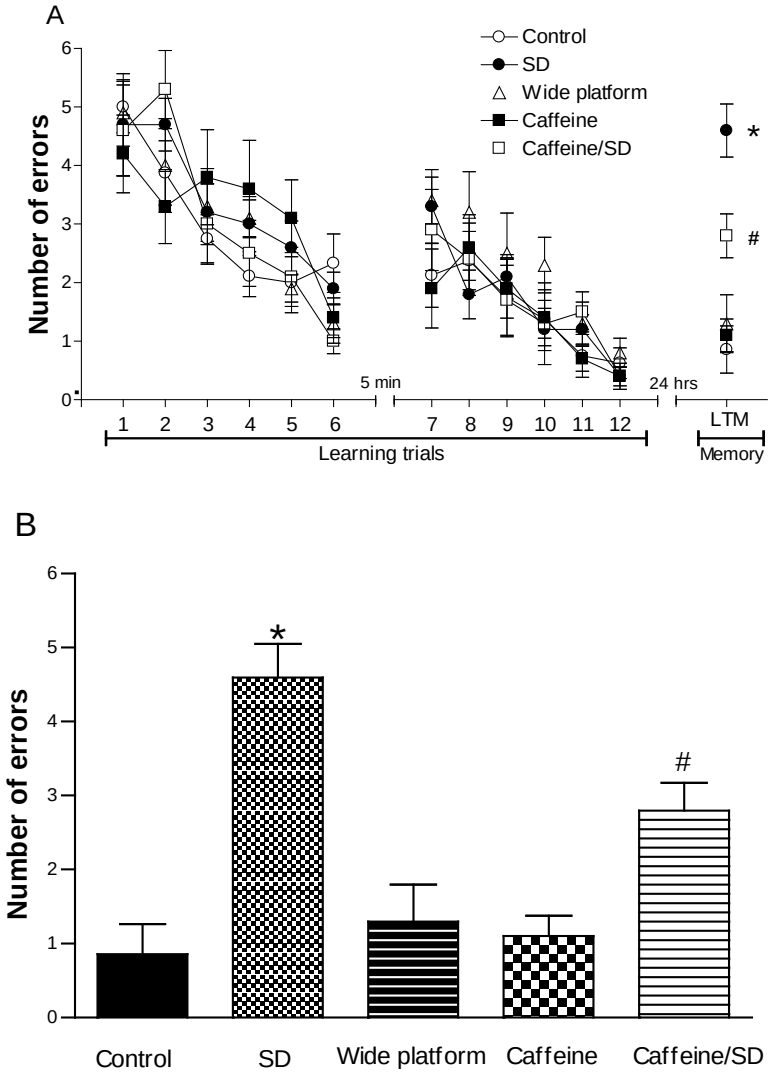


Figure 11: Effect of caffeine treatment on sleep deprivation-impaired long-term memory. (A) All groups performed similarly during learning phase (without sleep deprivation). Following 24 hr sleep deprivation, the sleep-deprived rats showed more errors in finding the hidden platform than control rats. Interestingly, chronic caffeine treatment partially prevented long-term memory impairment induced by 24 hr sleep deprivation. (B) Summarizes the average number of errors made by each of the 5 groups in long-term memory test. Each point is the mean ± SEM of 10-15 rats. * indicates significant difference from control and caffeine groups ($p < 0.01$) and # indicates significant difference from all other groups ($P < 0.05$).

4.2. Electrophysiological results

In this section, we assessed the effect of sleep deprivation and/or caffeine on synaptic function in the CA1 and DG areas before and after induction of long-term potentiation (LTP), which is widely accepted cellular model for learning and memory (Martin et al., 2000).

4.2.1. Electrophysiological results in CA1 area

4.2.1.1. Sleep deprivation does not affect basal synaptic function in CA1 area

To assess synaptic transmission in area CA1 synapses, the basal synaptic function was examined by applying a number of stimuli with increasing intensities to produce input-output (I-O) curves (stimulus intensity vs. field excitatory postsynaptic potential (fEPSP) slope in all four groups (fig. 12A). No significant differences were seen in the I/O relationship among the control, sleep deprivation, caffeine, and caffeine/sleep deprivation groups (fig. 12A). For example, at the maximum intensity, the mean fEPSP slope value was 6.47 ± 0.39 mV/ms for caffeine-treated rats, 6.46 ± 0.43 mV/ms for sleep-deprived rats, and 6.8 ± 0.24 mV/ms for caffeine/sleep deprivation rats which was statistically comparable ($P < 0.05$) to the mean fEPSP slope value for control rats (6.3 ± 0.8 mV/ms) (fig. 12A).

As an additional measure of basal synaptic function, we determined the mean stimulus intensities measured by voltage required to evoke minimum, maximum, and 30% of maximum responses. We found that the mean voltage values required for evoking fEPSP response in the three categories were statistically comparable in the control, sleep deprivation, caffeine, and caffeine/sleep deprivation groups (fig. 12B). These results indicate that neither sleep deprivation nor caffeine alters normal synaptic transmission in the CA1 area.

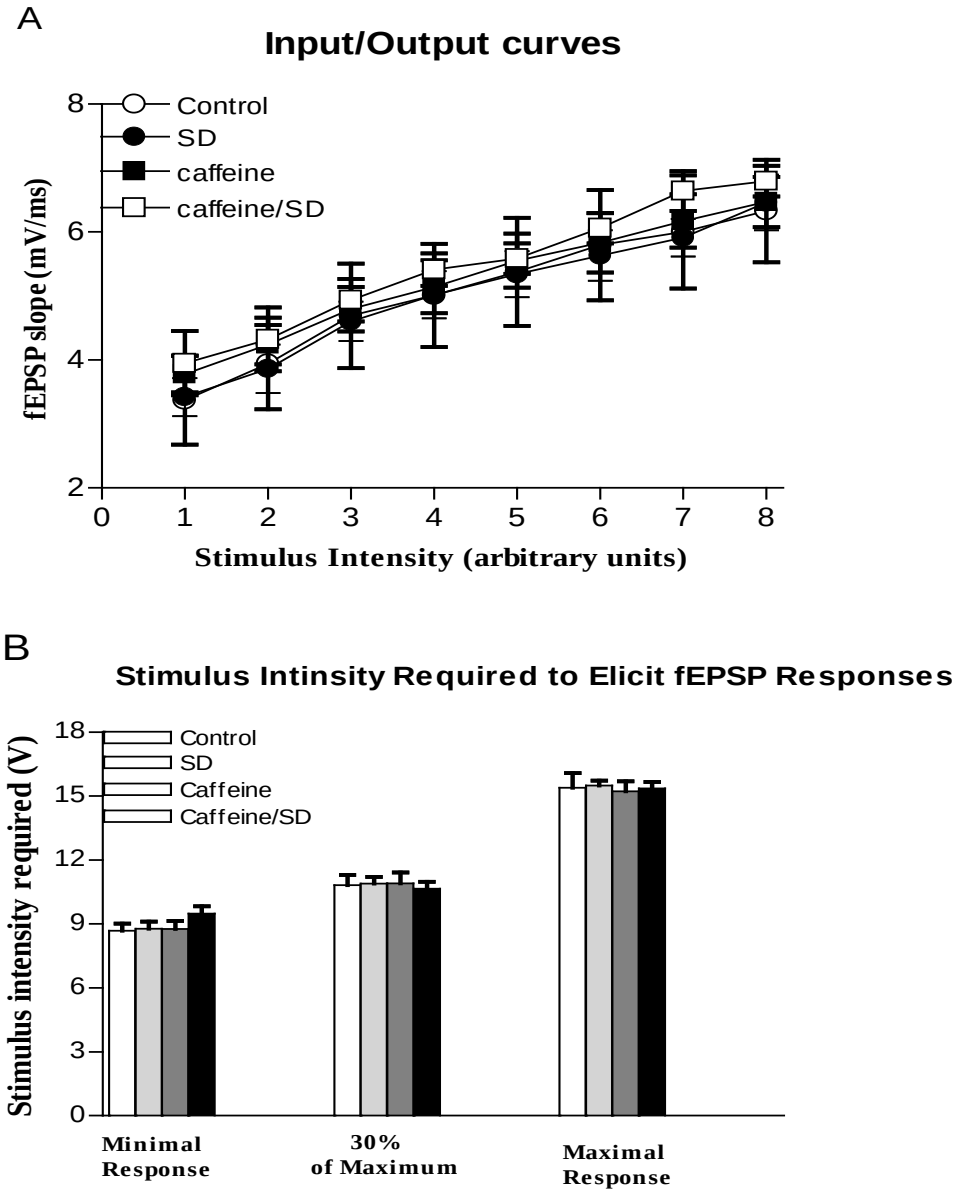


Figure 12: Basal synaptic transmission is normal in the CA1 area of the sleep-deprived rats. (A) input/output curves were obtained from responses to different stimulus intensities in the CA1 area. Stimulus intensity numbers are arbitrary units; where 1 is the intensity that generated minimum responses, and 8 is the intensity that generated maximum responses. (B) Stimulus intensity required to evoke the minimum, maximum, and 30% of the maximum response. Values are mean ± S.E.M. from 4-6 rats.

4.2.1.2. Chronic caffeine treatment prevents sleep deprivation-induced impairment of early phase long-term potentiation (E-LTP) in area CA1

Inasmuch as E-LTP is believed to be a cellular correlate of short-term memory and to confirm our behavioral data in which chronic caffeine treatment protects against short-term memory impairment induced by acute sleep deprivation, we next determined the impact of caffeine administration on the magnitude of E-LTP in sleep-deprived rats. The increase in the slope of the fEPSP (a measure of synaptic strength) and the pSpike amplitude (a measure for the number of neurons reaching threshold and firing action potentials) one hr after applying high frequency stimulation (HFS), were considered as indicators of E-LTP magnitude.

In the control group, applying HFS to CA3 area caused a robust increase in the fEPSP slope (t = 60 min: 157.15 ± 7.19% of baseline; fig. 13A) and amplitude of the pSpike (t = 60 min: 212.06 ± 13.32 % of baseline; fig. 13B) in area CA1. In sleep-deprived rats, although the slope of fEPSP (t = 60 min: 113.41 ± 4.9% of the baseline; fig. 13A) and the amplitude of the pSpike (t = 60 min: 119.14 ± 11.76% of the baseline; fig. 13B) were significantly ($P < 0.05$) higher than the baseline values, they were markedly lower ($F_{3,17} = 5.83$, $P = 0.0095$; $F_{3,17} = 12.78$, $P = 0.0005$, respectively) than those of rats in the control group.

In the caffeine/sleep deprivation group, the slope of fEPSP (151.86 ± 11.62% of the baseline; fig. 13A) and the pSpike amplitude (235.11 ± 8.3%; fig. 13B) were not significantly different than those of the control and caffeine groups measured one hr after applying the HFS. Thus, chronic caffeine treatment prevents sleep deprivation-induced impairment of E-LTP.

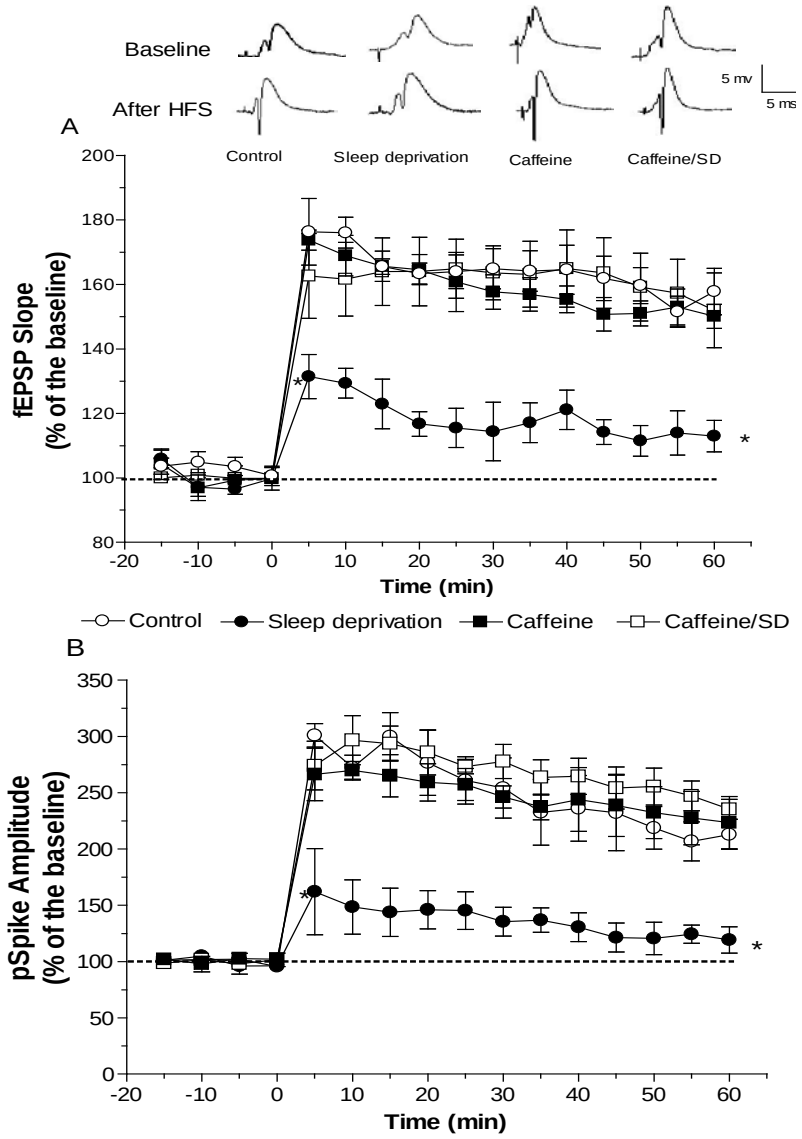


Figure 13**:** Early-phase long-term potentiation (E-LTP) of area CA1 evoked by high-frequency stimulation (HFS) at time zero, measured as increase in the slope of fEPSP and pSpike amplitude. (A) Chronic caffeine treatment prevents sleep deprivation-induced E-LTP impairment in the CA1 area. Sleep deprived rats exhibit markedly impaired slope of fEPSP, but chronic caffeine treatment prevents fEPSP slope impairment. (B) Chronic caffeine treatment before sleep deprivation prevents the decrease in pSpike amplitude. Each point is the mean ± SEM of 4-6 rats. All points between (*) are significantly different from other groups ($P < 0.05$). Inset is a representative experiment; calibrations, 5 mV/5 ms, apply to all traces.

4.2.1.3. Chronic caffeine treatment attenuates sleep deprivation-induced impairment of late phase long-term potentiation (L-LTP) in the CA1 area

The L-LTP is said to be a cellular correlate of long-term memory. To support our behavioral data in which chronic caffeine treatment prevented long-term memory deficit induced by acute sleep deprivation, we determined the impact of long-term caffeine administration on the magnitude of L-LTP decline in sleep-deprived rats.

Multiple high frequency stimulation (MHFS) of the Schaffer collateral pathway in area CA3 of the hippocampus evoked L-LTP indicated by a long-lasting increase in the slope of fEPSP (time = 300 min: 143.37 ± 8.25 % of baseline, $F_{3,21} = 4.43$, P = 0.0168; fig. 14A) and amplitude of pSpike (time = 300 min: 296.77 ± 59.10% of baseline, $F_{3,20} = 5.99$, P = 0.0056; fig. 14B) in area CA1 of the control group. Applying the same MHFS in the sleep deprivation group initially evoked a small, but significant, increase in the slope of fEPSP and the amplitude of pSpike. This enhancement gradually diminished back to the baseline level. The slope of fEPSP was 98.87 ± 9.29% of baseline (fig. 14A) and amplitude pSpike was 153.58 ± 19.05% of baseline (fig. 14B) five hrs after applying MHFS. Therefore, sleep deprivation impairs L-LTP, which appears to correlate with our behavioral results that suggest impairment of long-term memory by acute sleep deprivation.

Chronic caffeine treatment of normal rats did not change L-LTP magnitude, which was similar to that of control rats. In caffeine-treated sleep-deprived animals, the magnitude of L-LTP was similar to that of the control and caffeine groups but significantly higher than that of the sleep deprivation group. Five hours after MHFS, the slope of fEPSP (fig. 14A) and amplitude of pSpike (fig. 14B) were 131.12 ± 8.83% and 283.63 ± 10.69% of baseline, respectively, in the caffeine-treated sleep deprivation group. This clearly shows that long-term use of caffeine prevents sleep deprivation-induced impairment of L-LTP in the CA1 area of the hippocampus.

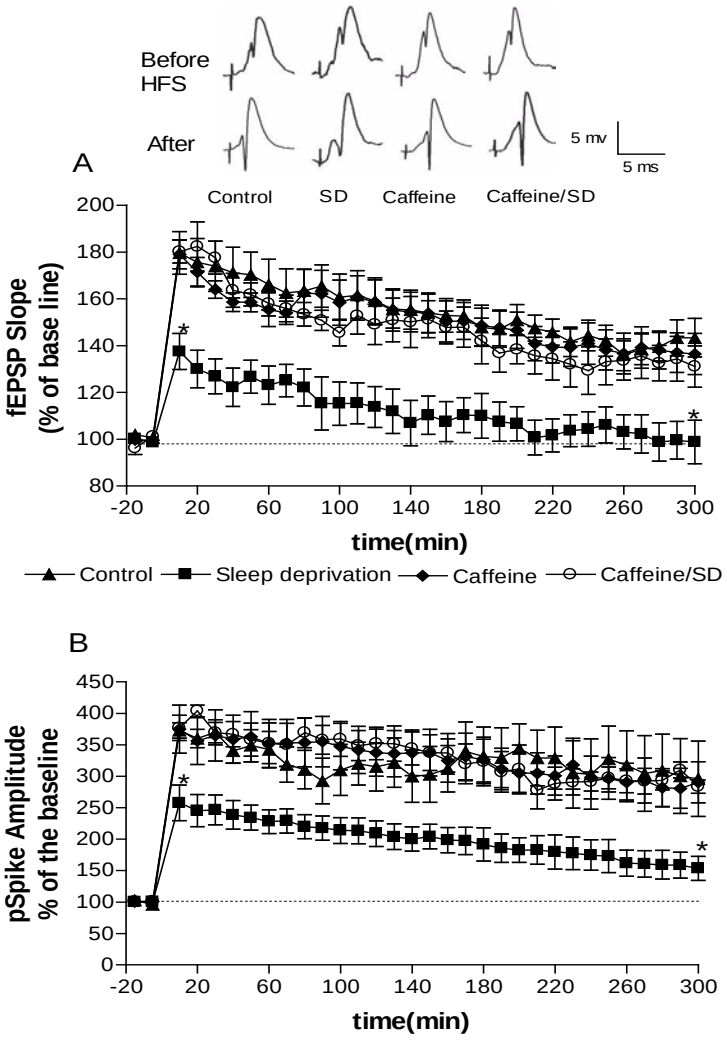


Figure 14: Late-phase long term potentiation (L-LTP) in the CA1 region of the hippocampus evoked by MHFS applied at time zero to the Schaffer collateral/commissural pathway of anesthetized rats. L-LTP was measured as increases in the slope of fEPSP and amplitude of pSpike expressed as percentage of the baseline values (before MHFS). (A) fEPSP slope values in the sleep-deprived rats are significantly lower than those of control, caffeine, and caffeine/sleep deprivation groups at all time point after applying MHFS, but chronic caffeine treatment prevents fEPSP slope suppression. (B) Chronic caffeine treatment prevents the sleep deprivation-induced reduction in pSpike amplitude. (*) Denotes significant difference from control, caffeine, and caffeine/sleep deprivation groups ($P < 0.05$, n = 4-6 rats/group). Insets are representative experiments; calibrations, 5 mV/5 ms, apply to all traces.

4.2.2. Electrophysiology studies in the DG area

4.2.2.1 Sleep deprivation does not affect basal synaptic function in DG area

Baseline pSpikes of the perforant path-DG synapses were recorded, before induction of E-LTP, by applying a series of increasing stimulus intensities to construct I/O curves. There was no significant difference in the I/O relationship represented by the slope of the f-EPSP among the control, sleep deprivation, caffeine, and caffeine/sleep deprivation groups (fig. 15A). Moreover, the required mean stimulus intensities to produce the minimum, 30% of maximum and maximum potentiation of fEPSP were not different in the four groups (fig. 15B). Therefore, basal synaptic transmission in the DG region seems to be unaltered by chronic caffeine treatment or 24 hr sleep deprivation.

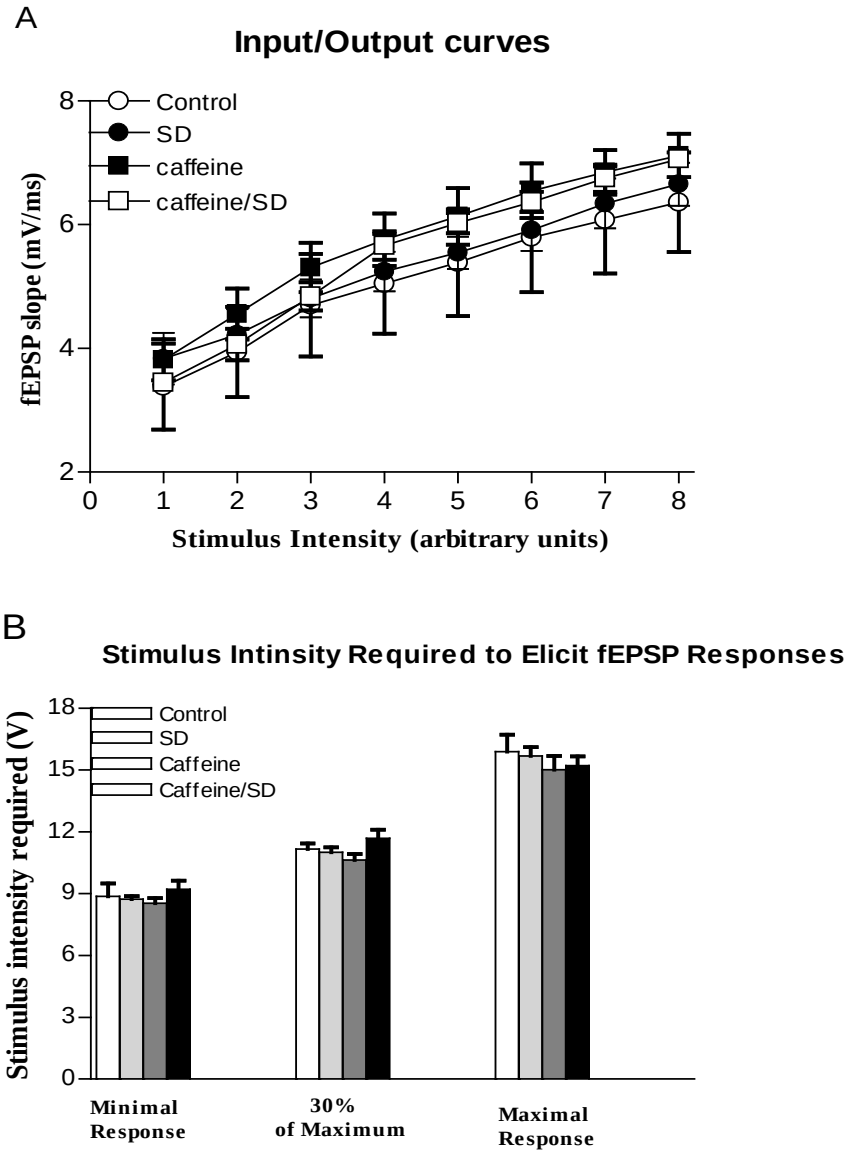


Figure 15: Basal synaptic transmission is normal in the DG area of the sleep-deprived rats. (A) input/output curves were obtained from recorded responses to different stimulus intensities in the DG area. Stimulus intensity numbers are arbitrary units; where 1 is the intensity that generated minimum responses, 8 is the intensity that generated the maximum responses. (B) Stimulus intensity essential to evoke the minimum, maximum, and 30% of the maximum response. Values are mean ± S.E.M. from 5-6 rats.

4.2.2.2. Chronic caffeine treatment prevents sleep deprivation-induced impairment of E-LTP in the DG area

We applied HFS to the angular bundle area to generate E-LTP in the DG area. In the control group, induction of LTP by HFS caused a marked increase in the fEPSP slope (t = 60 min: 154.16 ± 5.8% of baseline; fig. 16A) and amplitude of the pSpike (t = 60 min: 247.12 ± 7.37% of baseline; fig. 16B) in the DG area of the hippocampus. In contrast, in the sleep-deprived rats, the same stimulation procedure generated significantly ($P < 0.05$) less potentiation of the fEPSP slope (108.62 ± 6.23% of the baseline, $F_{3,21} = 12.79$, $P = 0.0001$; fig. 16A) and the amplitude of pSpike (130.59 ± 18.49% of the baseline, $F_{3,19} = 4.68$, $P = 0.0157$; fig. 16B) compared to the control group one hr after applying HFS.

In the caffeine-treated sleep deprivation group, the slope of fEPSP (t = 60 min: 156.17 ± 11.30% of the baseline; fig. 16A) and the amplitude of pSpike (t = 60 min: 260.71 ± 30.73%; fig. 16B) were not significantly different from those in the control or caffeine groups but were significantly different from the sleep deprivation group. Therefore, chronic caffeine intake prevents sleep deprivation-induced impairment of E-LTP in the DG area.

Since it is generally accepted that E-LTP is the cellular correlate of short-term memory, these results support our behavioral experiments, which suggest that chronic

caffeine treatment prevents sleep deprivation-induced impairment of cognitive ability in the short-term memory test.

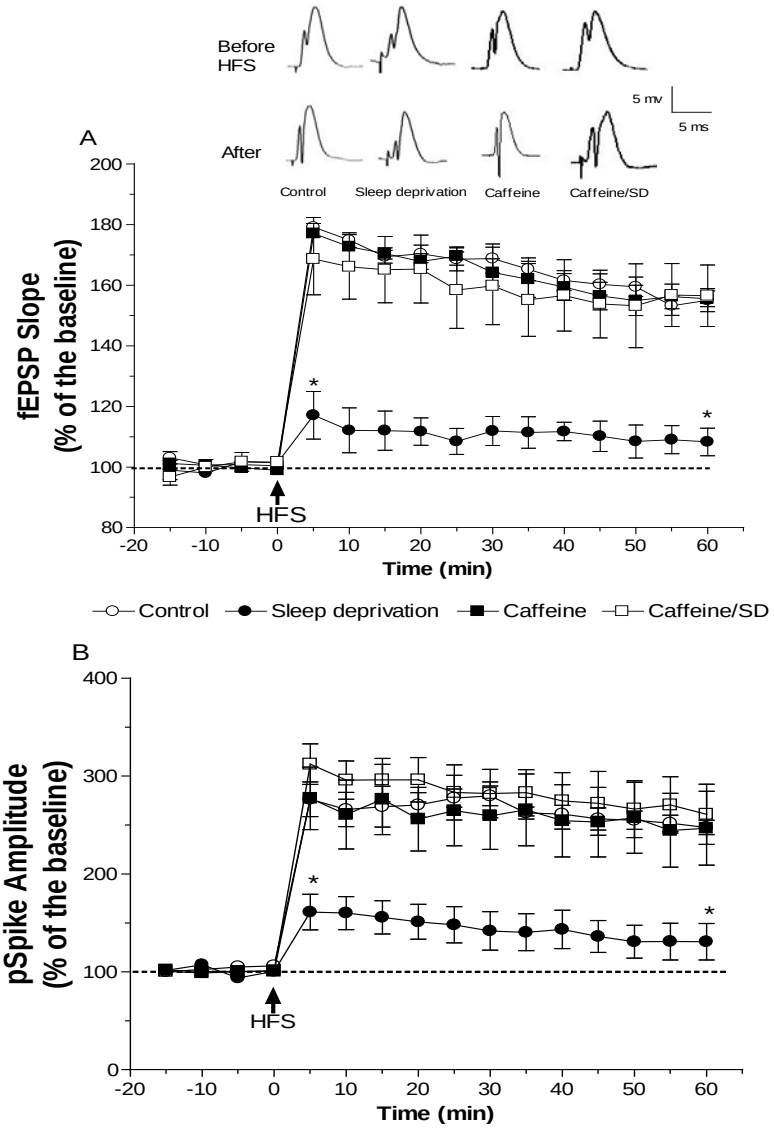


Figure 16: Hippocampal E-LTP in area DG evoked by HFS (at arrows) was measured as increase in the slope of fEPSP and pSpike amplitude. (A) Sleep-deprived rats exhibited markedly impaired slope of fEPSP, but chronic caffeine treatment prevents fEPSP slope impairment. (B) Chronic caffeine treatment prevents the sleep deprivation-induced reduction in pSpike amplitude. Each point is the mean ± SEM from 5-7 urethane-anesthetized rats. All the points between (*) are significantly different from other groups ($p < 0.05$). Insets are representative experiments; calibrations, 5 mV/5 ms, apply to all traces.

4.2.2.3. Chronic caffeine treatment attenuates sleep deprivation-induced impairment of L-LTP in the DG area

In the DG area of the hippocampus, we next examined L-LTP induced by MHFS of the perforant path. In control rats, L-LTP was induced as indicated by a long-lasting increase in the slope of fEPSP (t = 300 min: 140.31 ± 9.6% of baseline; fig. 17A) and amplitude of pSpike (247.48 ± 17.91% of baseline; fig. 17B), and was stably maintained for at least five hrs after applying MHFS. In contrast, the same stimulation procedure in the sleep deprivation group failed to generate potentiation of the fEPSP slope (107.01 ± 8.95% of the baseline, $F_{3,20}$ = 4.64, P = 0.0152) and the amplitude of pSpike (129.7 ± 22.54% of the baseline, $F_{3,17}$ =5.83, P = 0.0085) compared to the control group five hrs after applying MHFS.

In the caffeine-treated sleep deprivation group, the slope of fEPSP (141.36 ± 6.46% of the baseline; fig. 17A) and the amplitude of pSpike (271 ± 21.47%; fig. 17B) were not significantly different from those in the control or caffeine groups but were significantly ($P < 0.05$) different from the sleep deprivation group. Thus, chronic caffeine intake prevents sleep deprivation-induced impairment of L-LTP in the DG area.

The results of the effect of chronic caffeine treatment and/or sleep deprivation on E-LTP and L-LTP in CA1 and DG areas are summarized in table 2.

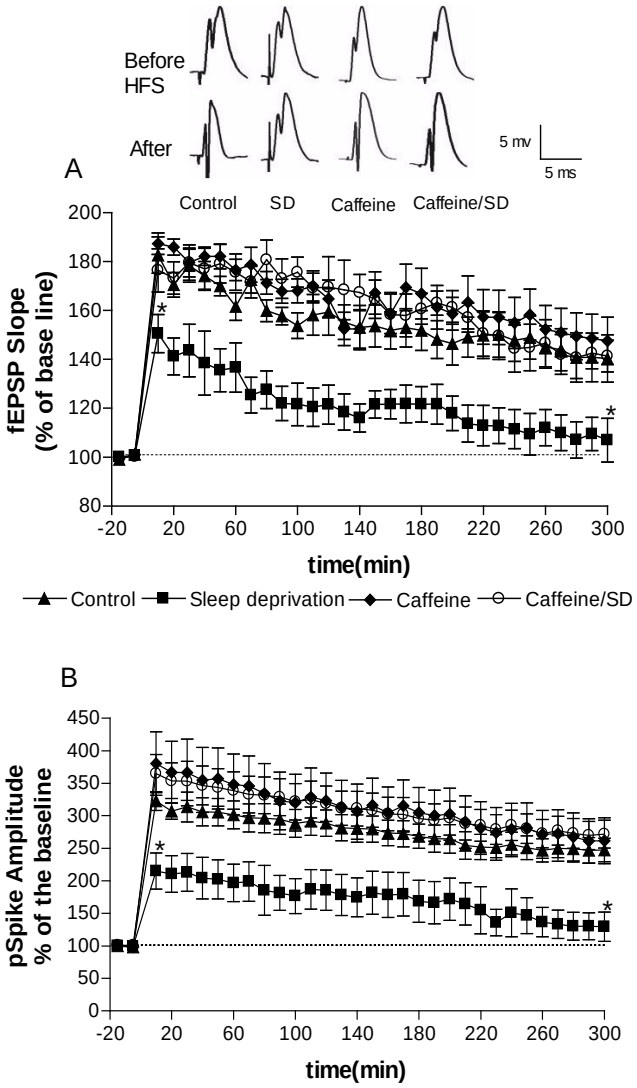


Figure 17: L-LTP in the DG region of the hippocampus evoked by MHFS applied at time zero to the perforant path of anesthetized rats. L-LTP was measured as increase in the slope of fEPSP and amplitude of pSpike expressed as a percentage of baseline values (before MHFS). (A) Slope values in sleep-deprived rats are significantly lower than those of control, caffeine, and caffeine/sleep deprivation groups at all time points after applying MHFS. (B) Chronic caffeine treatment prevents the sleep deprivation-induced reduction in pSpike amplitude. (*) Denotes significant difference from control, caffeine, and caffeine/sleep deprivation groups ($P < 0.05$, n = 4-6 rats/group). Insets are representative experiments; calibrations, 5 mV/5 ms, apply to all traces.

	E-LTP in CA1	E-LTP in DG	L-LTP in CA1	L-LTP in DG
Control	Normal	Normal	Normal	Normal
Sleep deprivation	Impaired	Impaired	Impaired	Impaired
Caffeine	Normal	Normal	Normal	Normal
Caffeine/sleep deprivation	Normal	Normal	Normal	Normal

Table 2: Summary of the effects of sleep deprivation and/or chronic caffeine treatment on both E-LTP and L-LTP in the CA1 and DG areas of the hippocampal formation.

4.3. Molecular results

Behavioral and electrophysiological results show that chronic caffeine administration prevents impairments of spatial memory and hippocampal-LTP in sleep-deprived rats. Thus, we wanted to know whether the basal levels of signaling proteins important for cognitive function and synaptic plasticity are altered as a result of sleep deprivation and/or chronic caffeine treatment in the CA1 and DG areas. In addition, we investigated the changes in the levels of these signaling molecules during the expression of E-LTP and L-LTP.

4.3.1 Molecular results in CA1 area

4.3.1.1. Basal levels of E-LTP-related signaling molecules

Since CaMKII, calcineurin, and BDNF are the major signaling molecules in the expression of E-LTP, we examined the impact of sleep deprivation and/or caffeine administration on the basal protein levels of CaMKII, calcineurin, and BDNF. We used the western blot method to measure levels in the total homogenate of CA1 and DG areas.

4.3.1.1.1 Basal protein levels of P-CaMKII and total CaMKII

Active CaMKII is widely accepted to be involved in spatial short-term memory and hippocampal-E-LTP (Malenka et al., 1989; Pettit et al., 1994; Thomas et al., 1994;

Lledo et al., 1995; Aleisa et al., 2006c). Inhibitors of CaMKII prevent LTP (Barria et al., 1997b), while CaMKII activators facilitate LTP (Pettit et al., 1994). Repetitive stimulation leads to a persistent increase in total (phosphorylated and non-phosphorylated) CaMKII in the hippocampus (Alzoubi et al., 2006). In sleep-deprived rats, the gene expression of CaMKII was significantly lower than that in control rats (Guzman-Martin et al. 2006).

Western blot analysis showed that sleep deprivation markedly reduced the basal levels of P-CaMKII, the active form of CaMKII, (0.81 ± 0.05) compared to the control value (1.21 ± 0.08, $F_{3,25}$ = 8.37, P = 0.0007; fig. 18A). Sleep deprivation also caused a marked decrease in the level of total CaMKII (0.91 ± 0.11) compared to the control value (1.42 ± 0.06, $F_{3,21}$ = 11.64, P = 0.0002; fig. 18B).

Long-term use of caffeine significantly increased the basal levels of total-CaMKII (1.8 ± 0.13), but had no effect on active CaMKII compared to control (fig. 18A, B). In addition, chronic caffeine treatment prevented the sleep deprivation-induced reduction in the basal levels of both total and P- CaMKII (fig. 18A, B). The ability of caffeine to prevent the reduction in the levels of P-CaMKII may explain the protective effect of caffeine on short-term spatial memory and hippocampal-LTP against sleep loss. The ratio of P-CaMKII to total CaMKII was unchanged in all the four groups, indicating a parallel decrease in the total-CaMKII and P-CaMKII (fig. 18C).

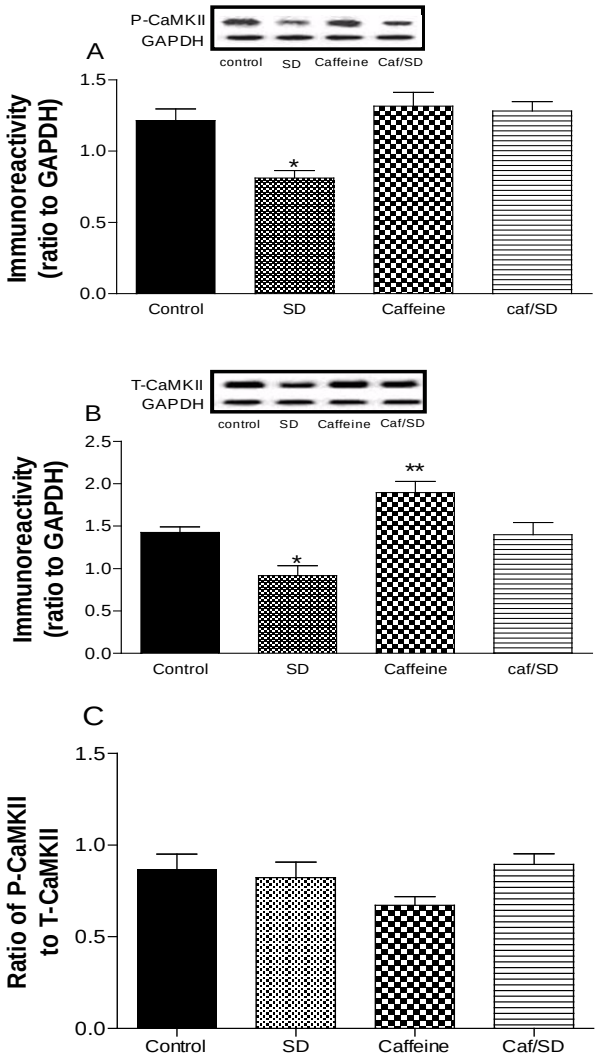


Figure 18: Basal levels of P-CaMKII and total-CaMKII in the CA area. Chronic caffeine treatment normalized sleep deprivation-induced decrease in the basal levels of P-CaMKII (A) and total CaMKII (B) in hippocampal CA1 region, measured in the total homogenate. No alteration in the P-CaMKII/CaMKII ratio is associated with sleep deprivation and/or caffeine (C). Values are mean ± SEM; n= 5-7. * Denotes significant difference from control, caffeine, and caffeine/sleep deprivation groups ($P < 0.05$). * Indicates significant difference from control group ($P < 0.05$). Insets are representative experiments.

4.3.1.1.2 Basal protein levels of calcineurin and brain derived neurotrophic factor (BDNF)

Considerable evidence indicates that calcineurin dephosphorylates P-CaMKII to its inactive form (Wang and Kelly, 1997). Previously, it has been reported in the hippocampus that sleep deprivation does not influence the levels of phosphatase-1, which is activated by calcineurin (Guan et al., 2004). We found that sleep loss for 24 hrs does not change the levels of calcineurin in the total homogenate of the CA1 area (fig. 19A).

Bain-derived neurotrophic factor (BDNF) plays an important role in hippocampal memory and LTP. For instance, mice lacking BDNF show reduction in hippocampal-LTP (Korte et al., 1995). It has been reported that sleep deprivation for 8 or 48 hrs reduces the levels of the BDNF in the hippocampus (Guzman-Marin et al., 2006). In agreement, the present results show that sleep loss for 24 hrs significantly ($P < 0.05$) reduced the basal levels of BDNF (0.56 ± 0.058) compared to the control values (0.91 ± 0.052) fig. 19B). Chronic treatment of sleep-deprived rats with caffeine normalized (rendered not significantly different from control) the BDNF basal levels (fig. 19B). In addition, there was no difference in the basal levels of BDNF between the caffeine and control groups.

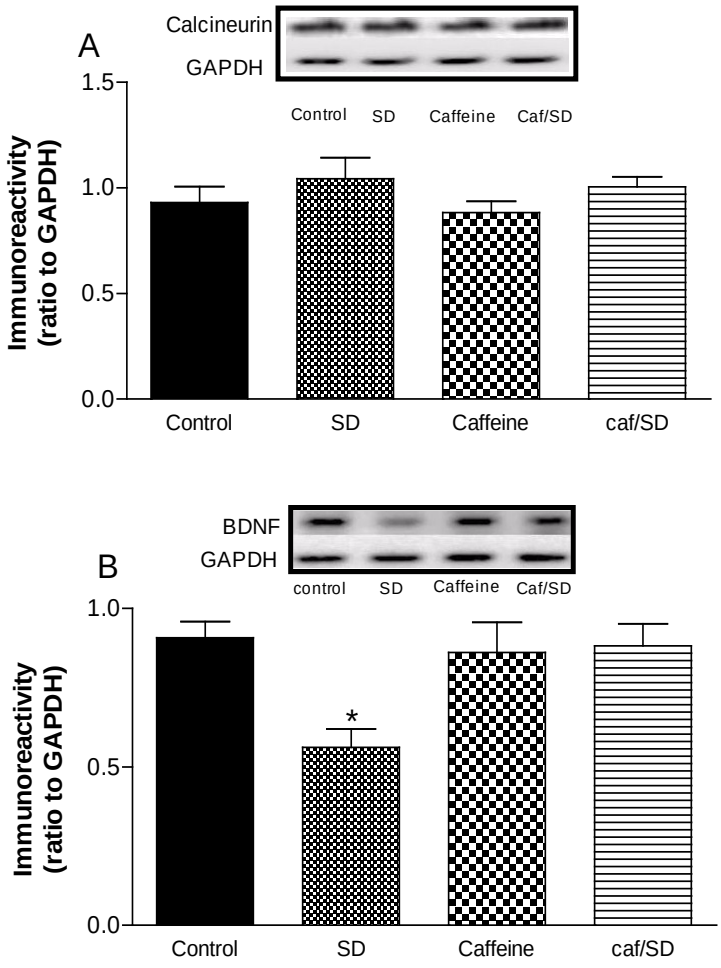


Figure 19: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of calcineurin (A) and BDNF (B); measured in area CA1 and expressed as a ratio to the GAPDH. Each point is the mean ± SEM of 4-6 rats. * Indicates significant difference from the control group ($P < 0.05$). Insets are representative experiments.

4.3.1.2. Levels of signaling molecules during the expression of E-LTP in the CA area

To reveal possible alterations in memory- and synaptic plasticity-related signaling molecules that may account for the reduction in the E-LTP expression, we measured protein levels of P-CaMKII, total-CaMKII, calcineurin, and BDNF one hr after induction of E-LTP. The temporal side was used as the internal control for the septal side in the same rat.

4.3.1.2.1. Levels of P-CaMKII and CaMKII during E-LTP

During the expression of E-LTP in area CA1, HFS produced similar increases ($P < 0.05$) in the protein levels of P-CaMKII in the stimulated (S)-control (1.46 ± 0.09), S-caffeine (1.46 ± 0.07) and S-caffeine/sleep deprivation (1.43 ± 0.05) groups but not in the S-sleep deprivation group (1.03 ± 0.08) compared to those of un-stimulated control (0.98 ±0.06, $F_{3,25} = 11.03$, $P < 0.01$; fig. 20A). Thus, it seems that the phosphorylation of CaMKII is impaired in the CA1 area of sleep-deprived rats. However, protein levels of total-CaMKII in all four stimulated groups were significantly ($P < 0.05$) increased to similar levels compared to the un-stimulated control group (fig. 20B). These findings suggest that sleep deprivation impairs the process of phosphorylation of CaMKII during E-LTP expression.

4.3.1.2.2. Levels of calcineurin and BDNF during E-LTP

The levels of calcineurin in area CA1 were markedly increased after the induction of E-LTP in all stimulated groups compared to the un-stimulated control group (fig. 21A), whereas BDNF protein levels, measured one hr after HFS in all 4 stimulated groups were not significantly different from those of the un-stimulated control (fig. 21B).

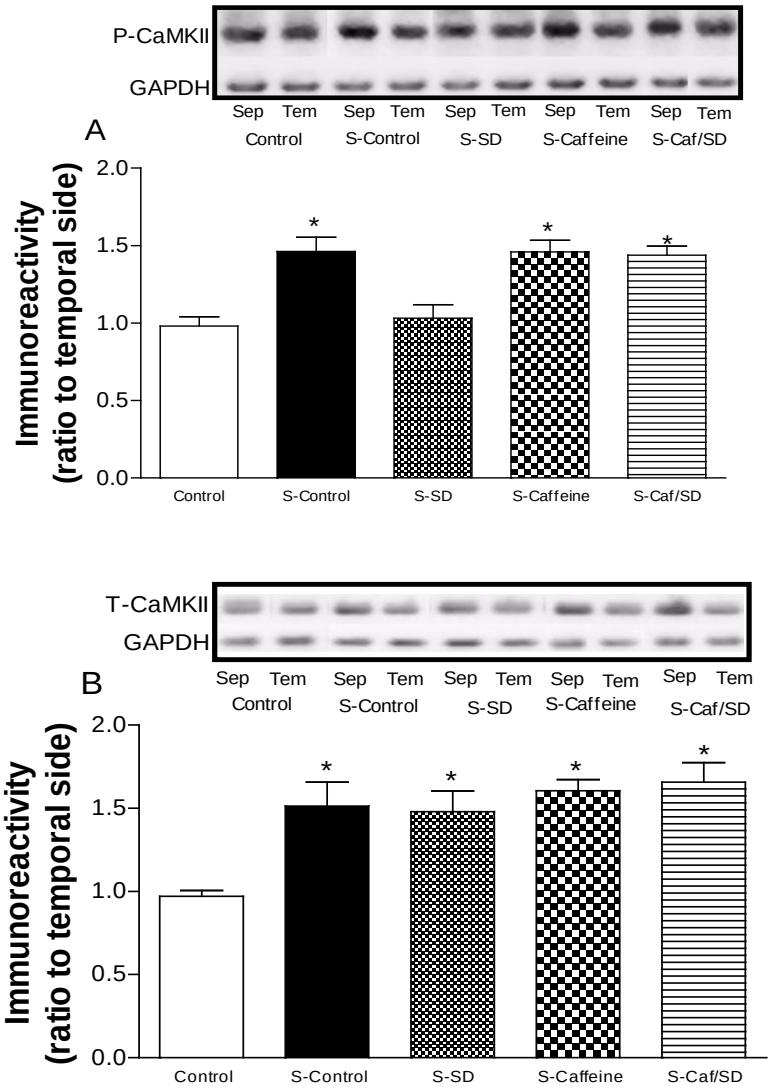


Figure 20: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of P-CaMKII (A) and total CaMKII (B) one hr after the induction of E-LTP, measured in the septal side of CA1 area and expressed as a ratio to the temporal side. S refers to stimulated groups. The temporal side serves as an "internal control" for the septal side of the hippocampus. To reduce individual variations, protein levels in the septal side were normalized as a percentage of those of the temporal side of the same hippocampus. Each point is the mean ± SEM of 5-6 rats. * indicates significant difference from un-stimulated control group ($P < 0.05$). Insets are representative experiments.

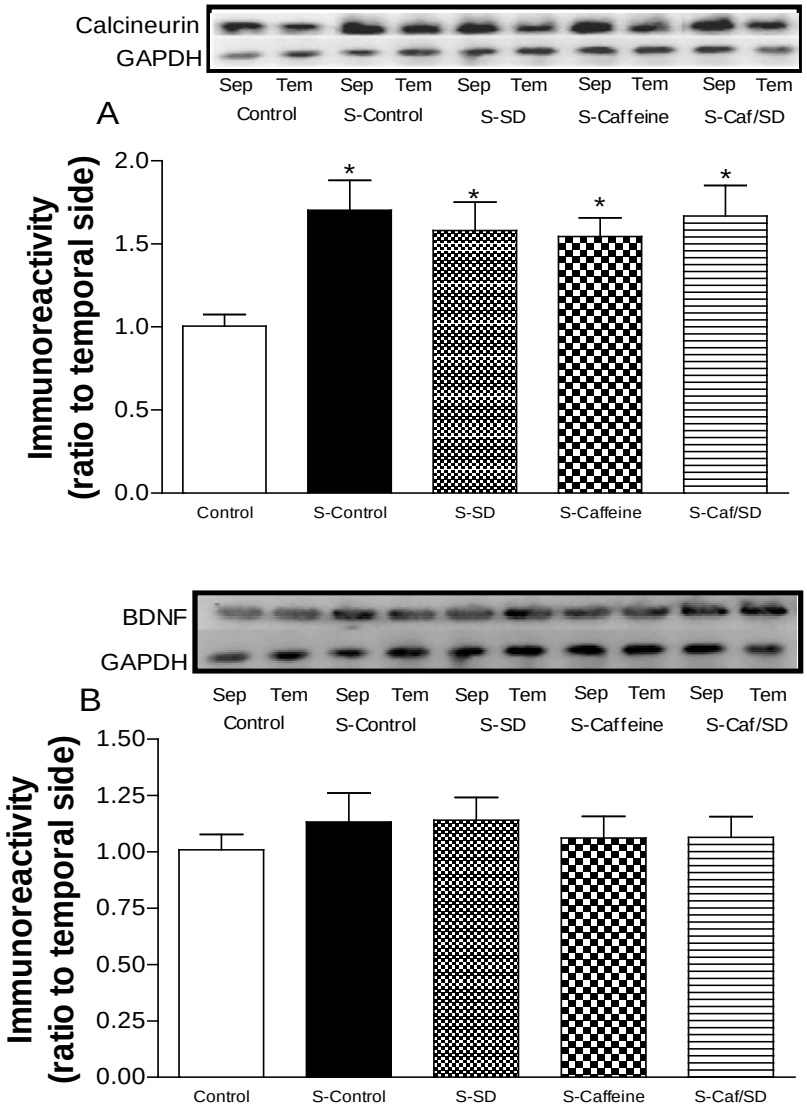


Figure 21: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of calcineurin (A) and BDNF (B) one hr after the induction of E-LTP; measured in the septal CA1 area and expressed as a ratio to the temporal side. S refers to the stimulated group. Each point is the mean ± SEM of 4-6 rats. * indicates significant difference from un-stimulated control group ($P < 0.05$). Insets are representative experiments.

4.3.1.3 Basal levels of L-LTP-related signaling molecules

It is widely accepted that cAMP response element binding protein (CREB) is the regulator of long-term memory and L-LTP. In this section, we examined the effects of sleep deprivation and/or caffeine administration on the protein levels of CREB and CaMKIV. We used the immunoblot method to measure the levels of CREB and CaMKIV in the total homogenate of CA1 and DG areas.

4.3.1.3.1 Basal levels of P-CREB and total-CREB in the CA1 area

Manipulation of CREB produces severe alterations in L-LTP (Bourtchuladze et al., 1994; Barco et al., 2002; Alarcon et al., 2004; Barco et al., 2005). Sleep loss for 8 hrs has been found to reduce the gene expression of CREB (Guzman-Marin et al., 2006). In the present study, we detected a significant reduction in the basal levels of P-CREB (0.31 ± 0.05, $P < 0.05$; fig. 22A) and total CREB (0.59 ± 0.04, $P < 0.05$; fig. 22B) in the CA1 area of sleep-deprived animals compared to control animals (P-CREB, 0.54 ± 0.05; T-CREB, 0.98 ± 0.11). This reduction could be responsible for sleep deprivation-induced long-term memory and L-LTP impairments in the CA1 region of the hippocampus.

Chronic caffeine treatment before sleep deprivation prevented the reduction in the levels of P-CREB and total-CREB in sleep-deprived rats. Levels of P-CREB and total-CREB were similar in control, caffeine, and caffeine-treated sleep deprivation rats (fig.

22 A, B). Furthermore, no alteration was observed in the ratio of P-CREB to total-CREB in all the groups (fig. 22C), which suggests that the P-CREB reduction associated with sleep deprivation could indicate an overall decrease in the total protein levels of CREB.

4.3.1.3.2 Basal levels of calcium/calmodulin-dependent protein kinase IV (CaMKIV)

CaMKIV is involved in the activation of CREB during the expression of L-LTP (Bito et al., 1996; Tokuda et al., 1997). Western blot analysis showed that sleep deprivation markedly reduced the basal levels of CaMKIV (0.86 ± 0.11) compared to the control value (1.37 ± 0.076; fig. 23). Long-term use of caffeine has no effect on protein levels of CaMKIV (1.36 ± 0.1; fig. 23). However, chronic caffeine treatment prevented the sleep deprivation-induced reduction in the basal levels of CaMKIV (1.42 ± 0.13; fig. 23). The ability of caffeine to prevent the reduction in the levels of CaMKIV may explain the protective effect of caffeine on the levels of active CREB.

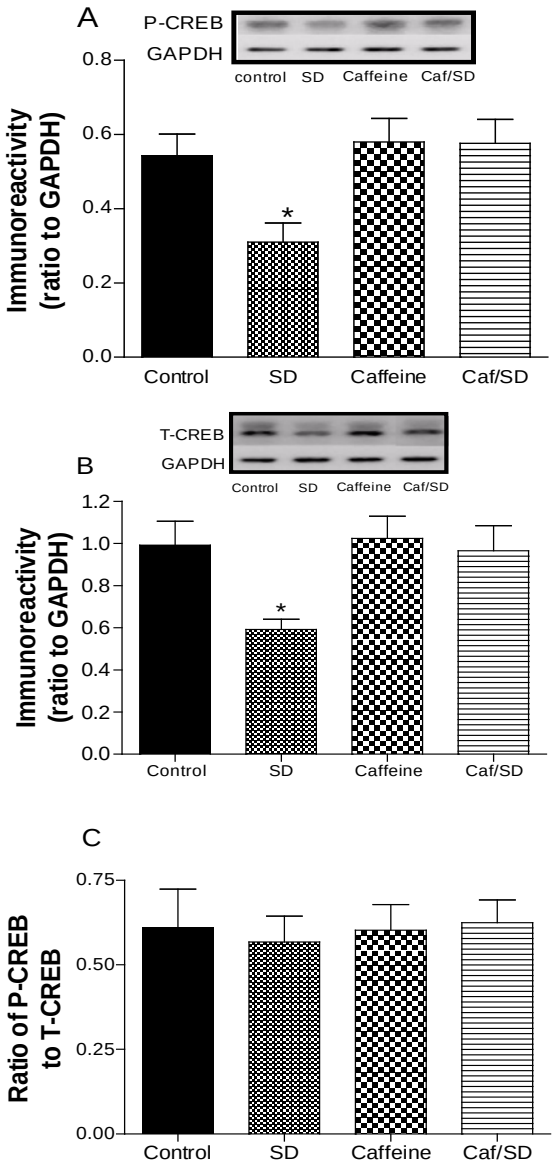


Figure 22: Chronic caffeine treatment normalized sleep deprivation-induced decrease in the basal levels of P-CREB (A) and total CREB (B) in hippocampal CA1 region, measured in the total homogenate. No alteration in the P-CREB/T-CREB ratio associated with sleep deprivation and/or caffeine (C). Values are mean ± SEM; n= 5-7. Insets are representative experiments. * indicates significant difference from control group ($P < 0.05$).

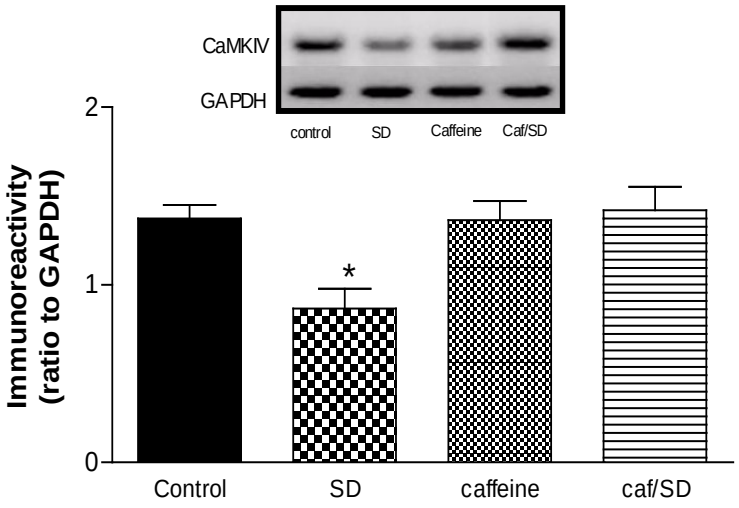


Figure 23: Chronic caffeine treatment normalized sleep deprivation-induced decrease in the basal levels of CaMKIV in hippocampal CA1 region, measured in the total homogenate. Values are mean ± SEM; n= 5-6. * indicates significant difference from control group ($P < 0.05$). Insets are representative experiments.

4.3.1.4. Levels of signaling molecules during the expression of L-LTP in CA1 area

To disclose possible changes in L-LTP-related molecules that may account for the ability of caffeine to prevent L-LTP impairment in sleep-deprived rats in the CA1 and DG areas, we determined the protein levels of P-CREB, total-CREB, CaMKIV, and BDNF five hrs after induction of L-LTP.

4.3.1.4.1 Levels of P-CREB and total-CREB during L-LTP

Phosphorylation and activation of CREB are essential for L-LTP and long-term memory (Bourtchuladze et al., 1994). Five hours after the induction of L-LTP in the CA1 area by MHFS, the levels of P-CREB were markedly ($P < 0.05$) increased in the S-control (1.5 ± 0.12) and S-caffeine (1.56 ± 0.12) groups but not in the S-sleep deprivation group (1.03 ± 0.08) compared to un-stimulated control (0.97 ± 0.05; fig. 24A). However, chronic caffeine treatment prevented the effects of sleep deprivation on the levels of P-CREB, which were increased (1.53 ± 0.1) in the caffeine/sleep deprivation group five hrs after MHFS (fig. 24A). No significant difference in the levels of P-CREB was observed among S-control, S-caffeine, and S-caffeine/sleep deprivation groups during L-LTP expression (fig. 24A). The protein levels of total-CREB in all stimulated groups, including the sleep deprivation group, were markedly ($P < 0.05$) increased compared to the un-stimulated control group (fig. 24B). Therefore, the decline in the CREB phosphorylation process may account for the sleep deprivation-induced reduction of L-LTP in the CA1 area.

4.3.1.4.2. Levels of CaMKIV during L-LTP

Five hours after the induction of L-LTP in the CA1 area by MHFS, the levels of CaMKIV were markedly ($P < 0.05$) increased in all stimulated groups, including the sleep deprivation group compared to the un-stimulated control group (fig. 25B).

4.3.1.4.3. Levels of BDNF during L-LTP

The BDNF gene, which is a target of CREB (Tao et al., 1998), plays an essential role in the persistence of long-term memories (Bekinschtein et al., 2008). In the current study, the protein levels of BDNF were markedly ($P < 0.05$) increased in the S-control (1.6 ± 0.09) and S-caffeine (1.53 ± 0.17) groups five hrs after the induction of L-LTP (fig. 26). However, no increase in BDNF levels was observed in sleep-deprived rats (1.07 ± 0.07), which were not significantly different from those of basal control (1.01 ± 0.07). In caffeine-treated sleep-deprived rats, levels of BDNF were increased (1.53 ± 0.86) to an extent similar to those of the S-control and S-caffeine groups (fig. 26). The increase in BDNF levels was significantly ($P < 0.05$) higher in the S-control, S-caffeine, and S-caffeine/sleep deprivation rats compared to those in S-sleep-deprived rats.

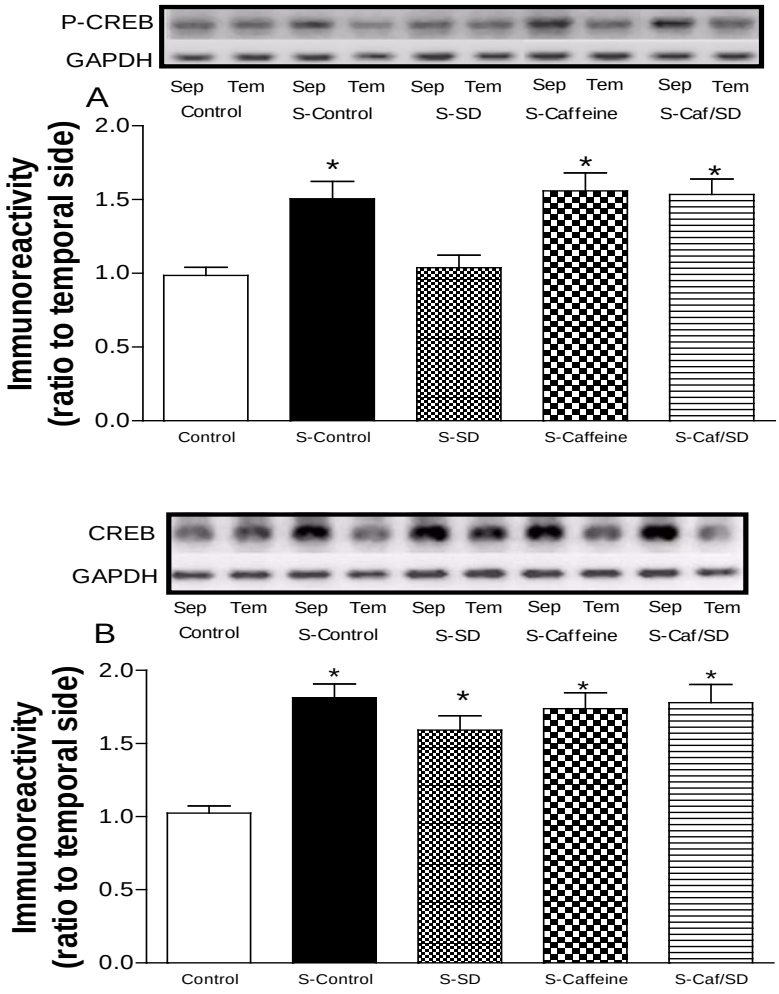


Figure 24: Effect of chronic caffeine treatment and/or sleep deprivation on the levels of CREB (phosphorylated and total) in the hippocampal CA1 area during L-LTP. (A) MHFS stimulation of the Schaffer collaterals synapses in the hippocampus caused a significant increase in the levels of phosphorylated (P)-CREB in the CA1 of stimulated (S)-control, s-caffeine, and S-caffeine/sleep deprivation groups but not in the S-sleep deprivation animals. (B) The levels of CREB in all stimulated groups were significantly increased compared to the un-stimulated control group. The temporal side serves as an "internal control" for the septal side of the hippocampus. To reduce individual variations, protein levels in the septal side were normalized as a percentage of those of the temporal side of the same hippocampus. * Indicates significant difference ($P < 0.05$, n = 5-6 rats/group) from control values. Insets are bands from representative experiments.

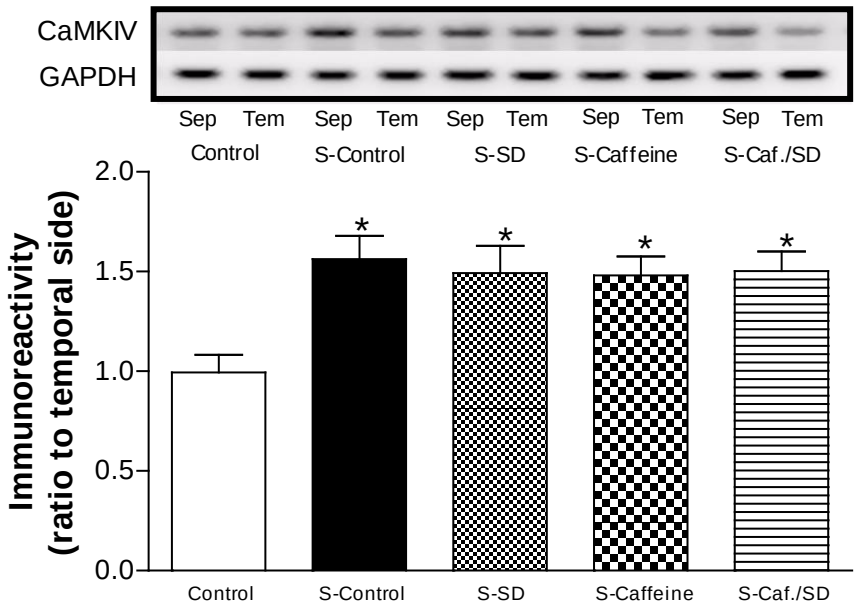


Figure 25: Effect of chronic caffeine treatment and/or sleep deprivation on the levels of CaMKIV in the hippocampal CA1 area during L-LTP. The levels of CaMKIV in all stimulated groups were significantly increased compared to the un-stimulated control. The temporal side serves as an "internal control" for the septal side of the hippocampus. * Indicates significant difference ($P < 0.05$, n = 4-7 rats/group) from control values. Insets are bands from representative experiments.

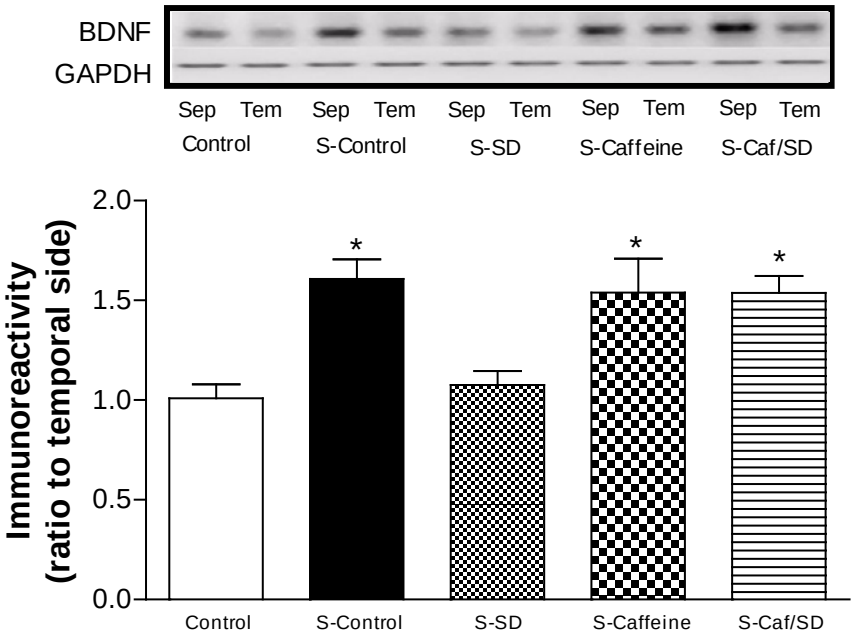


Figure 26: BDNF levels during L-LTP in the CA1 region of the hippocampus. The levels of BDNF after MHFS are significantly increased in all stimulated groups except the sleep deprivation group compared to the un-stimulated control group. * Indicates significant difference ($P < 0.05$, n = 4-5 rats/group) from control values. Insets are bands from representative experiments.

4.3.2. Molecular studies in the DG area

4.3.2.1. Basal levels of E-LTP-related signaling molecules in DG area

4.3.2.1.1 Basal protein levels of P-CaMKII and total CaMKII

The basal protein levels of active CaMKII (P-CaMKII) in the DG region in the sleep deprivation group (0.61 ± 0.1) were markedly decreased ($P < 0.05$) compared to that of the control group (1.13 ± 0.06), whereas, they were unchanged in the caffeine and caffeine/sleep deprivation groups (fig. 27A). The decrease in the P-CaMKII levels may account for the short-term memory and LTP impairment associated with sleep deprivation. Moreover, the basal protein levels of total-CaMKII (phosphorylated and non-phosphorylated forms) in sleep-deprived rats (0.92 ± 0.07) were also markedly decreased ($P < 0.05$) compared to the control rats (1.46 ± 0.08; fig. 27B). In contrast, chronic caffeine treatment increased the basal levels of total-CaMKII (1.86 ± 0.05) compared to control (fig. 27B). Caffeine treatment normalized the basal levels of total-CaMKII in sleep-deprived rats. In addition, we found no change in the ratio of P-CaMKII to total CaMKII levels (fig. 27C) in all experimental groups. These results are similar to these seen in the CA1 region.

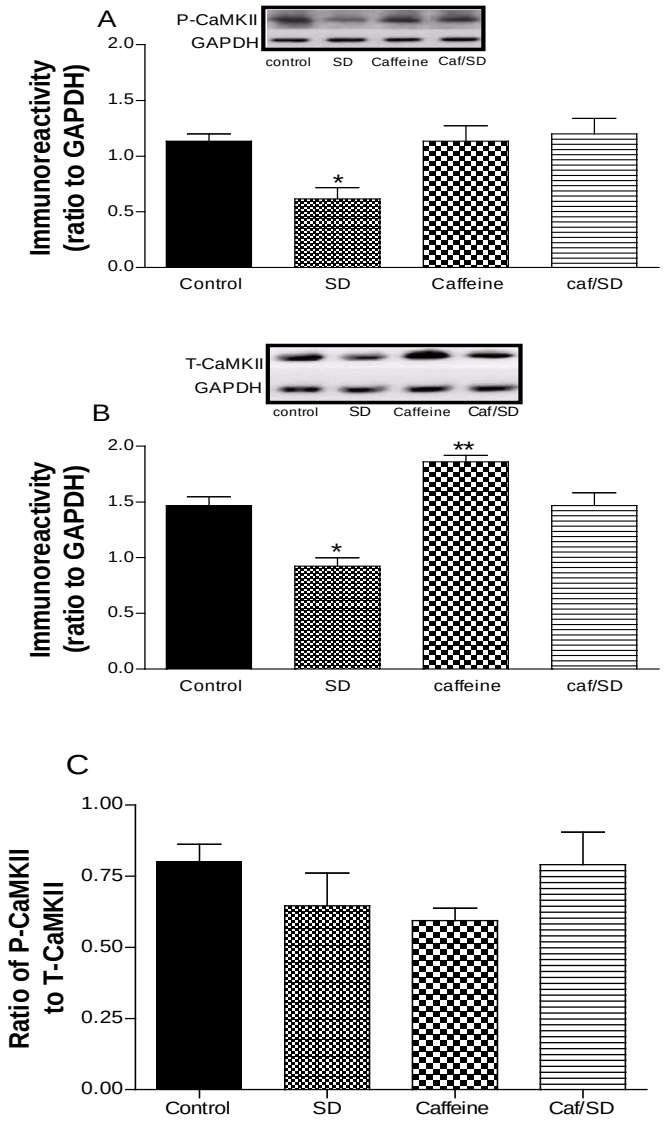


Figure 27: Chronic caffeine treatment normalized the sleep deprivation-induced decrease in the basal levels of P-CaMKII (A) and total CaMKII (B) in hippocampal DG region, measured in the total homogenate. No alteration in the P-CaMKII/CaMKII ratio is associated with sleep deprivation and/or caffeine (C). Values are mean ± SEM; n= 5-7. Insets are representative experiments. * Denotes significant difference from control, caffeine, and caffeine/sleep deprivation groups ($P < 0.05$). * Indicates significant difference from control group ($P < 0.05$).

4.3.2.1.2 Basal protein levels of calcineurin and BDNF

In this section, we assessed the effect of 24 hr of sleep loss on the basal levels of calcineurin in the DG region and found that there was no difference in the calcineurin levels among the four experimental groups (fig. 28A).

The present results show that sleep deprivation for 24 hrs decreased the basal levels of BDNF (0.35 ± 0.05) compared to the control value (0.62 ± 0.06; fig.28B). Even though chronic caffeine treatment did not affect the basal levels of BDNF, it prevented the sleep deprivation-induced decrease in the levels of BDNF in the caffeine/sleep deprivation group (fig. 28B).

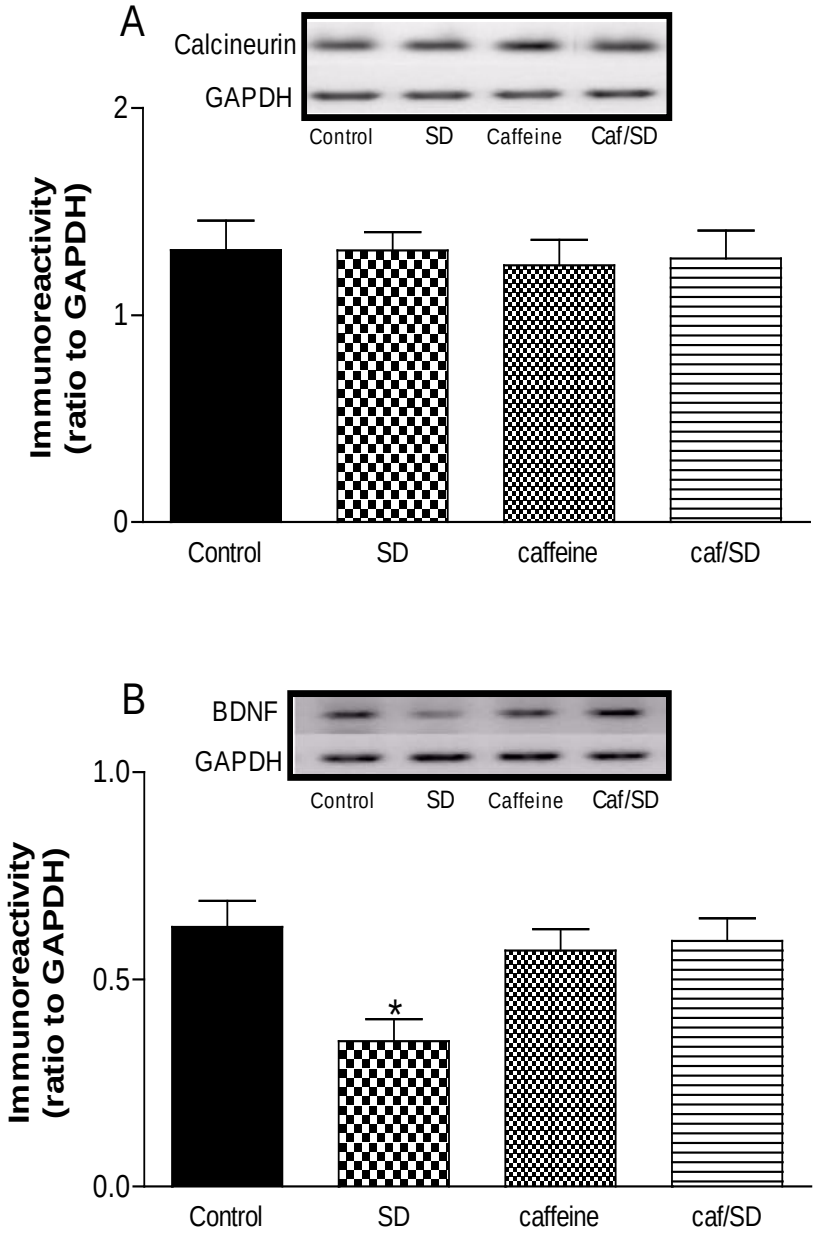


Figure 28: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of calcineurin (A) and BDNF (B); measured in area DG and expressed as a ratio to the GAPDH. Each point is the mean ± SEM of 4-6 rats. * Indicates significant difference from control group ($p < 0.05$). Insets are representative experiments.

4.3.2.2. Levels of signaling molecules during the expression of E-LTP in the DG area

4.3.2.2.1. Levels of P-CaMKII and CaMKII during E-LTP

One hour after the induction of E-LTP in the DG area by HFS, the levels of P-CaMKII were markedly ($P < 0.05$) increased in the S-control (1.58 ± 0.15) and S-caffeine (1.57 ± 0.1) groups, respectively, but not in the S-sleep deprivation group (1.04 ± 0.09) compared to un-stimulated control (0.9 ± 0.05; fig. 29A). However, chronic caffeine treatment prevented the effects of sleep deprivation on the levels of P-CaMKII, which were increased in the caffeine/sleep deprivation group (1.56 ± 0.1) one hr after HFS (fig. 29A). No significant difference in the levels of P-CaMKII was observed among the S-control, S-caffeine, and S-caffeine/sleep deprivation groups during LTP expression (fig. 29A). The protein levels of total-CaMKII in all stimulated groups, including the sleep deprivation group, were markedly ($P < 0.05$) increased compared to the un-stimulated control group (fig. 29B). Therefore, the decline in the phosphorylation process of CaMKII may account for the sleep deprivation-induced reduction of E-LTP in the DG area.

4.3.2.2.2. Levels of calcineurin and BDNF during E-LTP

During the expression of E-LTP in the DG area**,** the levels of calcineurin were significantly ($P < 0.05$) enhanced after the induction of E-LTP in the stimulated control,

caffeine, sleep deprivation, and caffeine/ sleep deprivation groups compared to the un-stimulated group (fig. 30A).

The induction of LTP by using HFS produced no significant alterations in the levels of BDNF in all four stimulated groups compared to the un-stimulated control group in the DG region (fig. 30B).

A summary of the effects of HFS on the levels of P-CaMKII, total-CaMKII, calcineurin, and BDNF, on all groups, in the total homogenate of CA1 and DG areas is presented in table 3.

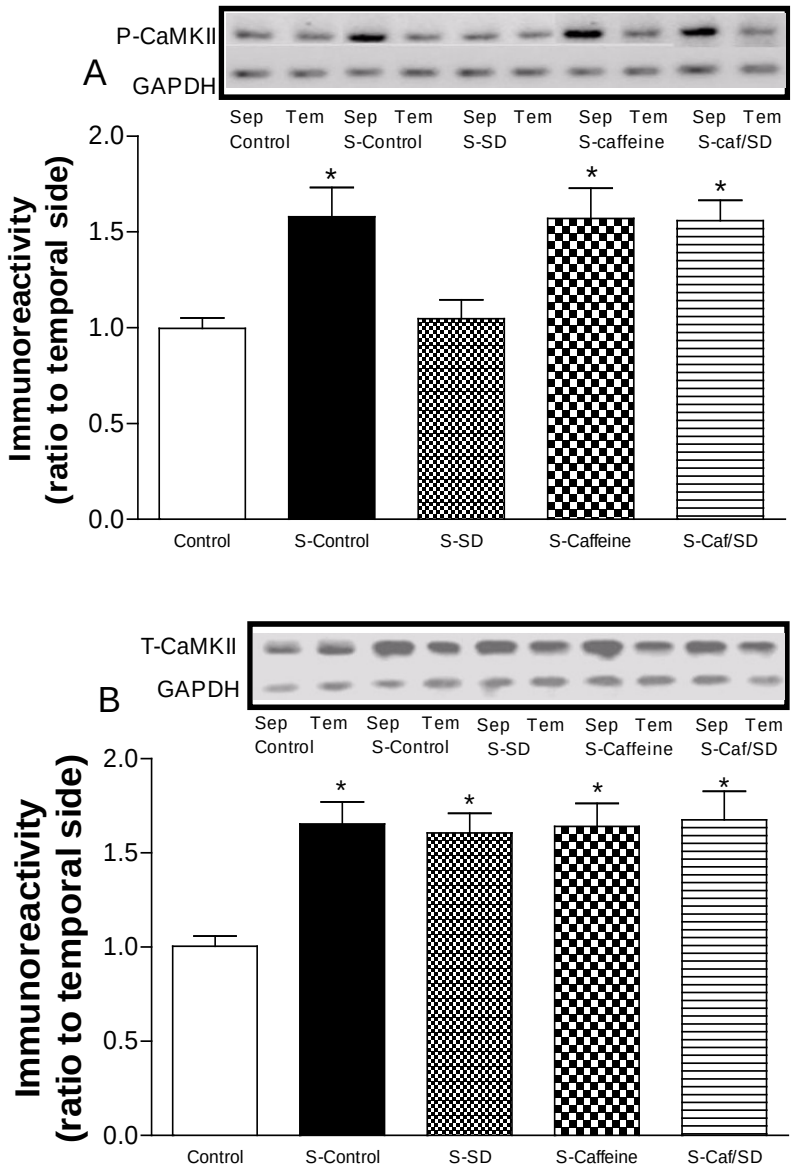


Figure 29: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of P-CaMKII (A) and total CaMKII (B) one hr after the induction of E-LTP, measured in the septal side of DG area and expressed as a ratio to the temporal side. S refers to stimulated groups. Each point is the mean ± SEM of 5-6 rats. * indicates significant difference from un-stimulated control group ($P < 0.05$). Insets are representative experiments.

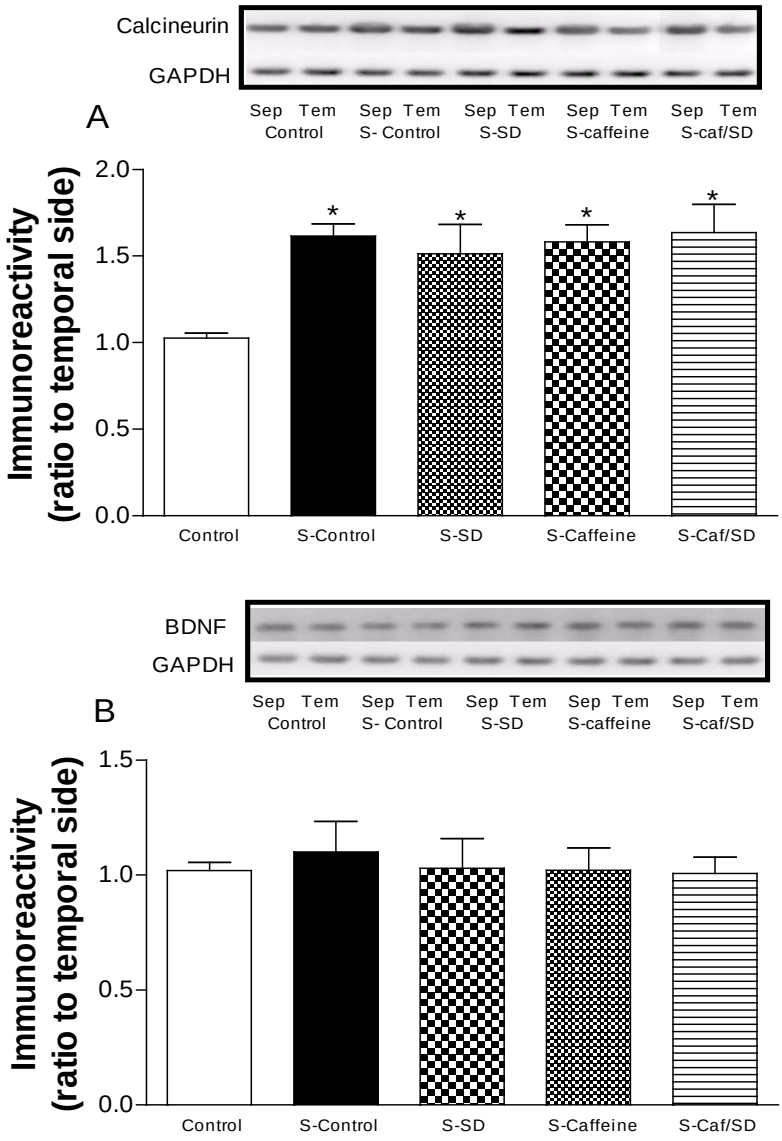


Figure 30: Effect of chronic caffeine treatment and/or sleep deprivation on the protein levels of calcineurin (A) and BDNF (B) one hr after the induction of E-LTP; measured in the septal DG area and expressed as a ratio to the temporal side. S refers to the stimulated group. Each point is the mean ± SEM of 4-6 rats. * indicates significant difference from un-stimulated control group ($P < 0.05$). Insets are representative experiments.

	S-control	S-sleep deprivation	S-caffeine	S-caffeine/sleep deprivation
P-CaMKII	Increase	No change	Increase	Increase
Total-CaMKII	Increase	Increase	Increase	Increase
Calcineurin	Increase	Increase	Increase	Increase
BDNF	No change	No change	No change	No change

Table 3: Summary of the effects of HFS on the levels of signaling molecules during the expression of E-LTP. Protein levels of P-CaMKII, total-CaMKII, calcineurin, and BDNF in the septal side of the right CA1 or DG area of the hippocampus compared to the corresponding temporal side in control, sleep deprivation, caffeine, and caffeine/sleep deprivation groups compared to the un-stimulated control group. (S-) indicates stimulated.

4.3.2.3. Basal levels of signaling molecules related to L-LTP in the DG area

4.3.2.3.1. Basal levels of P-CREB and total-CREB

The basal protein levels of the active CREB in the DG region in the sleep deprivation group (0.3 ± 0.05) were markedly decreased ($P < 0.05$) compared to those in the control group (0.6 ± 0.06), whereas, they were unchanged in the caffeine (0.59 ± 0.08) and caffeine/sleep deprivation (0.58 ± 0.04) groups (fig. 31A). The decrease in the P-CREB levels may account for L-LTP impairment caused by sleep deprivation in the DG area. Moreover, the basal protein levels of total-CREB in sleep-deprived rats (0.59 ± 0.06) were also markedly decreased ($P < 0.05$) compared to those in control rats (1 ± 0.15; fig. 31B). Caffeine treatment normalized the basal levels of total-CREB in sleep-deprived rats. In addition, we found no change in the ratio of P-CREB to total CREB levels (fig. 31C) in all experimental groups.

4.3.2.3.2. Basal levels of CaMKIV

The basal protein levels of CaMKIV in sleep-deprived rats (0.97 ± 0.097) were markedly decreased ($P < 0.05$) compared to the control rats (1.54 ± 0.093; fig. 32). Although, chronic caffeine treatment did not change the levels of CaMKIV (1.53 ± 0.11) in the total homogenate of DG area, it did prevent the decrease of this molecule (1.61 ± 0.14) in acutely sleep-deprived rats.

Summary of the effects of chronic caffeine treatment and/or sleep deprivation on the basal levels of P-CaMKII, total-CaMKII, ratio of P-CaMKII to total-CaMKII, calcineurin, BDNF, P-CREB, total-CREB, ratio of P-CREB to total-CREB, and CaMKIV in all groups, in the total homogenate of CA1 as well as DG area are presented in table 4.

	sleep deprivation	**Caffeine**	**caffeine/sleep deprivation**
P-CaMKII	Decrease	No change	No change
Total-CaMKII	Decrease	Increase	No change
P-CaMKII:T-CaMKII	No change	No change	No change
Calcineurin	No change	No change	No change
BDNF	Decrease	No change	No change
P-CREB	Decrease	No change	No change
Total-CREB	Decrease	No change	No change
P-CREB:T-CREB	No change	No change	No change
CaMKIV	Decrease	No change	No change

Table 4: Summary of the effects of chronic caffeine treatment and/or sleep deprivation on the basal levels of signaling molecules important for E-LTP and L-LTP expression. Protein levels of P-CaMKII, total-CaMKII, ratio of P-CaMKII to total-CaMKII, calcineurin, BDNF, P-CREB, total-CREB, ratio of P-CREB to total-CREB, and CaMKIV in the CA1 or DG area of the hippocampus of sleep-deprived, caffeine, and caffeine-treated sleep deprivation rats are compared to those of the control rats.

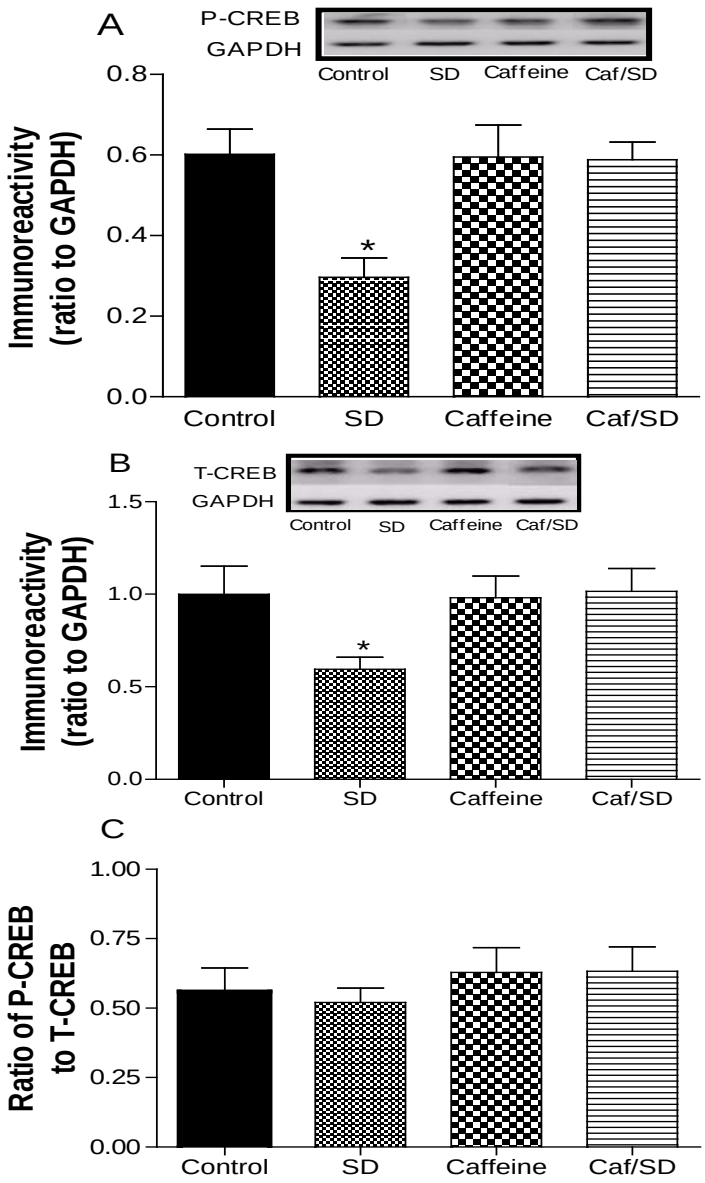


Figure 31: Chronic caffeine treatment normalized the sleep deprivation-induced decrease in the basal levels of P-CREB (A) and total CREB (B) in hippocampal DG region, measured in the total homogenate. No alteration in the P-CREB/CREB ratio associated with sleep deprivation and/or caffeine (C). Values are mean ± SEM; n= 5-7. Insets are representative experiments. * indicates significant difference from un-stimulated control group ($P < 0.05$).

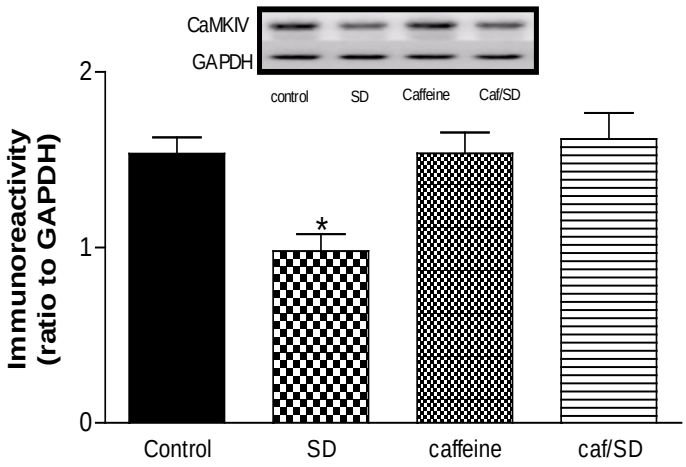


Figure 32: Chronic caffeine treatment normalized the sleep deprivation-induced decrease in the basal levels of CaMKIV in hippocampal DG region, measured in the total homogenate. Values are mean ± SEM; n= 5-7. Insets are representative experiments. * indicates significant difference from control group ($P < 0.05$).

4.3.2.4. Levels of signaling molecules during the expression of L-LTP in the DG area

4.3.2.4.1. Levels of P-CREB and total-CREB during L-LTP

Five hrs after the induction of L-LTP in the DG area of the hippocampus by MHFS, the levels of P-CREB in the DG area were markedly increased (approximately 1.54 ± 0.11, and 1.66 ± 0.12: S-control, S-caffeine animals, respectively, but not in the S-sleep-deprived animals (1.05 ± 0.08) compared to the un-stimulated animals (1.02 ± 0.07; fig. 33A). Chronic caffeine treatment prevented the negative effects of sleep deprivation on the levels of P-CREB, which were increased (1.53 ± 0.15) in the caffeine/sleep deprivation group five hrs after MHFS (fig. 33A). No significant difference in the levels of P-CREB was detected in the total homogenate among S-control, S-caffeine, and S-caffeine/sleep deprivation groups during L-LTP expression (fig. 33B).The levels of total-CaMKII were significantly increased to a similar extent in all stimulated groups (by approximately 50.71%, 56.01%, 59.26%, and 47.66%: S-control, S-sleep deprivation, S-caffeine, and S-caffeine/sleep deprivation groups, respectively) five hrs after the induction of L-LTP compared to the un-stimulated control group (fig. 33B).

4.3.2.4.2. Levels of CaMKIV during L-LTP.

The levels of CaMKIV were significantly increased to a similar extent in all stimulated groups (approximately 1.59 ± 0.1, 1.57 ± 0.13, 1.5 ± 0.17, and 1.58 ± 0.11: S-control, S-sleep deprivation, S-caffeine, and S-caffeine/sleep deprivation groups, respectively) five

hrs after the induction of L-LTP compared to the un-stimulated control group (1.04 ± 0.05; fig. 34).

4.3.2.4.3. Levels of BDNF during L-LTP.

The levels of BDNF were significantly ($P < 0.05$) increased in the S-control, S-caffeine, and S-caffeine/sleep deprivation animals (approximately 1.58 ± 0.14, 1.54 ± 0.08, and 1.5 ± 0.11, respectively compared to basal control 1.04 ± 0.08) after the induction of L-LTP in the DG area of the hippocampus (fig. 35). On the other hand, sleep loss for 24 hrs did not change the levels of BDNF (1.03 ± 0.05) five hrs after L-LTP induction compared to the un-stimulated control group.

A summary of the effects of MHFS on the levels of P-CREB, total-CREB, CaMKIV, and BDNF, on all groups, in the total homogenate of CA1 or DG area is presented in table 5.

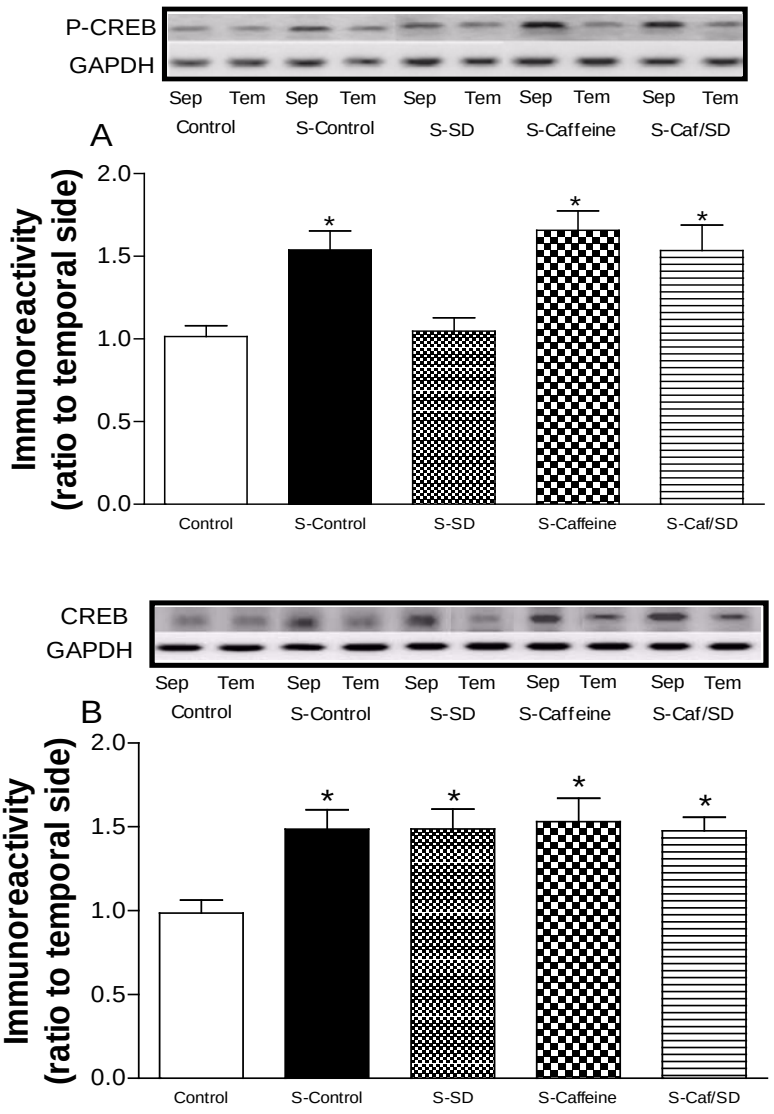


Figure 33: Effect of chronic caffeine treatment and/or sleep deprivation on the levels of CREB (phosphorylated and total) in the hippocampal DG area during L-LTP. (A) MHFS stimulation of the perforant path synapses in the hippocampus caused a significant increase in the levels of phosphorylated (P)-CREB in the DG of stimulated (S)-control, S-caffeine, and S-caffeine/sleep deprivation groups but not in the S-sleep deprivation animals. (B) The levels of CREB in all stimulated groups were significantly increased compared to the un-stimulated control. * Indicates significant difference ($P < 0.05$, n = 5-6 rats/group) from control values. Insets are bands from representative experiments.

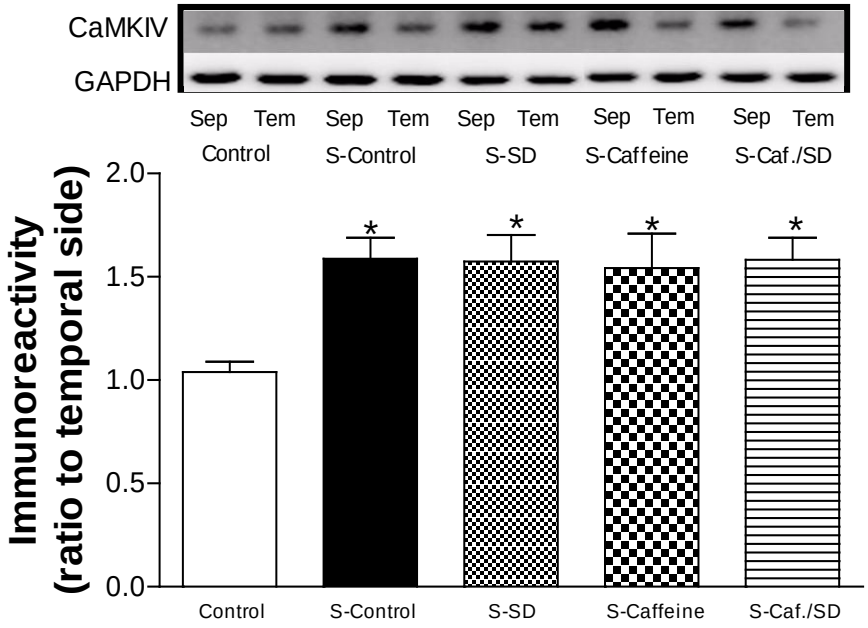


Figure 34: Effect of chronic caffeine treatment and/or sleep deprivation on the levels of CaMKIV in the hippocampal DG area during L-LTP. The levels of CaMKIV in all stimulated groups were significantly increased compared to the un-stimulated control. * Indicates significant difference ($P < 0.05$, n = 5-7 rats/group) from control values. Insets are bands from representative experiments.

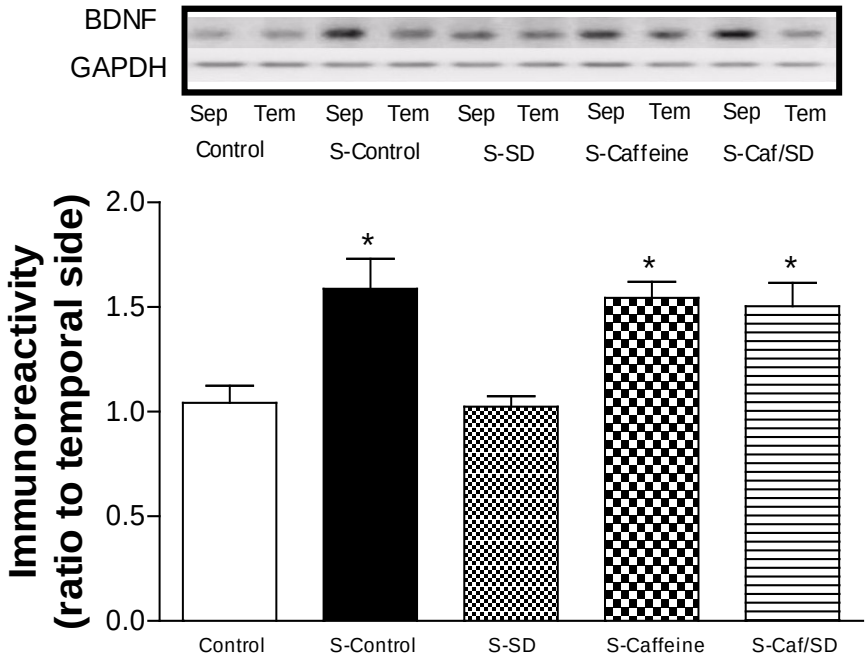


Figure 35: BDNF levels during L-LTP in the DG region of the hippocampus. The levels of BDNF after MHFS are significantly increased in all stimulated groups except the S-sleep deprivation group compared to the un-stimulated control group. * Indicates significant difference ($P < 0.05$, n = 5-6 rats/group) from control values. Insets are bands from representative experiments.

	S-control	S-sleep deprivation	S-caffeine	S-caffeine/sleep deprivation
P-CREB	Increase	No change	Increase	Increase
Total-CREB	Increase	Increase	Increase	Increase
CaMKIV	Increase	Increase	Increase	Increase
BDNF	Increase	No change	Increase	Increase

Table 5: Summary of the effects of MHFS on the levels of signaling molecules during the expression of L-LTP. Protein levels of P-CREB, total-CREB, CaMKIV, and BDNF in the septal side of the CA1 or DG area of the hippocampus compared to the corresponding temporal side in control, sleep deprivation, caffeine, and caffeine/sleep deprivation groups compared to the un-stimulated control group. (S-) indicates stimulated.

5. DISCUSSION

Numerous studies have shown that caffeine and sleep deprivation produce positive and negative effects, respectively, on learning and memory. However, the combined effect of caffeine and sleep deprivation on hippocampus-dependent learning and memory has not been investigated. In this study, we tested the effect of chronic caffeine treatment on memory impairment induced by sleep deprivation using three experimental approaches, behavioral, electrophysiological, and molecular. Our findings demonstrate that the chronic use of a low dose of caffeine protects against sleep deprivation-induced learning, short-term memory, and long-term memory impairments. This is revealed by the ability of chronic caffeine treatment before sleep deprivation to normalize the performance of sleep-deprived rats to the level of control rats in the radial arm water maze (RAWM). Moreover, chronic caffeine treatment prevents the sleep deprivation-induced deficit in E-LTP and L-LTP, which are believed to be the cellular correlates of short-term and long-term memory, respectively, in both the CA1 and DG areas. Analysis of the levels of signaling molecules by western blot demonstrates that chronic caffeine treatment attenuates the decrease in the basal protein levels of P-CaMKII, total-CaMKII, and BDNF in the CA1 area as well as the DG area in sleep-deprived rats. Additionally, the failure of HFS to increase the levels of P-CaMKII in the sleep deprivation group is averted by caffeine administration. In the CA1 and DG areas, the

decrease in the basal protein levels of CREB and CaMKIV in acutely sleep-deprived rats is prevented by caffeine treatment. Levels of P-CREB are increased five hrs after MHFS in the control, caffeine, and caffeine/sleep deprivation groups, but not in the sleep deprivation group, which may suggest that P-CREB is responsible for caffeine-induced protection against L-LTP impairment associated with 24 hr sleep deprivation.

5.1 The columns-in-water model

The harmful effect of sleep loss on cognition has been demonstrated regardless of the model of sleep deprivation. In our study, we sleep-deprived rats by using the modified multiple platform model, which depends on the loss of muscle tone during REM sleep. This model produces a marked decrease (90-95%) in rapid eye movement (REM) sleep, which is similar to the methods that use electroencephalographic recording to sleep-deprived subjects (Datta et al., 2004; Machado et al., 2004). The modified multiple platforms method has obvious advantages over other models of sleep deprivation. This paradigm allows rats from the same cage to be sleep-deprived together as a group to maintain established social hierarchy and remove possible isolation stress associated with the single and multiple flowerpot techniques. In addition, rats can move freely from one platform to another, which eliminates the immobilization stress associated with the single flowerpot technique (van Hulzen and Coenen, 1981; Suchecki et al., 1998; Rechtschaffen and Bergmann, 2002; Machado et

al., 2004). Moreover, some methods such as head lifting and gentle handling require continuous monitoring by the investigator, which may limit the time for sleep deprivation (Vogel, 1975; Vyazovskiy et al., 2002b; Datta et al., 2004). The moving disc and treadmill techniques involve forced physical activity (Stefurak et al., 1977; Guzman-Marin et al., 2003), which interferes with LTP in the hippocampus (O'Callaghan et al., 2007).

5.2. Behavioral experiments

The behavioral model used in this study (radial arm water maze) is a combination of the radial arm maze (RAM) and the Morris water maze (MWM). The radial arm water maze (RAWM) maintains the advantages of both RAM and MWM while curtailing their drawbacks (Buresova et al., 1985; Hodges, 1996; Diamond et al., 1999; Alamed et al., 2006). For instance, RAWM combines the spatial complexity of the RAM with the efficient learning of the MWM. Furthermore, the structure of the RAWM forces the animals to swim either in the central open area or in the arms, which abolishes the major disadvantage of the MWM, that is, swimming around the walls. Given that food is not required in the RAWM, the consequence of smell cues in the RAM is eradicated. Conditions that harmfully affect hippocampal functions such as aging (Buhot et al., 2003), high fat diet (Alzoubi et al., 2009b), chronic stress (Aleisa et al., 2006d), Alzheimer's disease (AD) (Srivareerat et al., 2008), epilepsy (Karnam et al., 2009),

hypothyroidism (Alzoubi et al., 2009a), and combination of stress and beta-amyloid peptides (Tran et al., 2010) have been shown to impair functioning in the RAWM.

5.2.1 Sleep deprivation and memory

Several lines of evidence from animal and human studies suggest that sleep deprivation adversely affects functions of the central nervous system; it particularly impairs the ability to retain new information and disrupts memory consolidation (Dubiela et al., 2010 ; Youngblood et al., 1999; McDermott et al., 2003; Guan et al., 2004; Ruskin et al., 2004; Yoo et al., 2007; Yang et al., 2008; Gohar et al., 2009; Mograss et al., 2009). The present study revealed that 24 hr sleep deprivation before training interfered with spatial learning and short-term memory tests and that acute sleep deprivation after training impaired long-term memory in the radial arm water maze. In agreement with our results, the majority of reports demonstrated cognitive impairment with sleep deprivation through using a variety of learning and memory tests including the radial arm maze (Smith et al., 1998), Morris water maze (Youngblood et al., 1999; Li et al., 2009; Wang et al., 2009), contextual fear conditioning (Graves et al., 2003), eight-box task (Bjorness et al., 2005), and novel arm recognition task (Hagewoud et al., 2009). However, some reports showed that sleep deprivation had no effects on memory function (Samkoff and Jacques, 1991; Blissitt, 2001), which may be attributed to

differences in the type of memory tested, complexity of the tasks, stage of sleep, duration of sleep loss, and the experimental protocols employed.

The present behavioral results suggest that the cognitive ability of rats kept on the wide platforms in the aquarium for 24 hr is not significantly different than that of the home-cage control rats. This may indicate that any possible stress resulting from being in the aquarium environment is not a causative factor for memory impairment seen in rats sleep-deprived on the narrow modified multiple platforms. In support of this conclusion, rats tested for 72 hrs on the wide platform did not show elevation in the level of the stress hormone corticosterone, compared to that of the home-cage control rats (Mirescu et al., 2006). In the same study, these authors reported that 24 hr of sleep deprivation in the narrow platform did not change the levels of corticosterone. Additionally, rats sleep-deprived in the modified multiple narrow platform method for 96 hrs and then immediately treated with metyrapone, an inhibitor of corticosterone synthesis, showed a marked memory deficit (Tiba et al., 2008). These results may indicate that stress, if any, does not mediate memory impairment in the modified multiple platform method of sleep deprivation.

It is possible that the deficit in performance in the RAWM may be due to factors other than direct effects of sleep deprivation including impaired motor function, stress (repeatedly falling into the water) and related disorders such as anxiety. Although we

did not measure the swim speed of rats, there were no obvious signs of impaired ability to swim among the groups. Stress may contribute to the cognitive deficits seen in the electrophysiological and molecular studies, but we believe that sleep deprivation is the major contributor. This is because stress influences the hippocampus in a substantially different way than sleep deprivation. Previous reports from this laboratory show that, unlike sleep deprivation, stress does not affect learning or long-term memory (Aleisa et al., 2006d), and nor does it impair DG function (Gerges et al., 2001).

5.2.2. Caffeine and memory

Caffeine is implicated in the modulation of learning and memory functions probably by virtue of its action as a non-selective adenosine receptors antagonist (Kopf et al., 1999). There is some debate as to whether caffeine plays a positive or negative role, if any, in cognitive function. While some investigators report an improvement of cognition as a consequence of caffeine treatment (Roussinov and Yonkov, 1976; Warburton, 1995; Riedel and Jolles, 1996; Hameleers et al., 2000; Angelucci et al., 2002; Costa et al., 2008b; Capek and Guenther, 2009; Foskett et al., 2009; Smith, 2009), others report no effect (Furusawa, 1991; Hudzik and Wenger, 1993; Herz, 1999; Warburton et al., 2001). Still others report deterioration of memory after caffeine treatment (Terry and Phifer, 1986; Sansone et al., 1994; Fisher and Guillet, 1997)

In the present experiments, caffeine did not affect learning, short-term memory, and long-term memory in control rats. These contradictory results regarding the effect of caffeine on learning and memory may be due to differences in the length of the treatment, dosage form and dose level of caffeine, stage of memory tested, experimental protocol used, or time of administration. The caffeine dose-response curve is an inverted U, which shows that at low doses (0.3-3 mg/kg), caffeine improves memory consolidation, whereas at high doses (30-100 mg/kg), it disrupts the acquisition phase of the water maze reference task, which measures hippocampus-dependent memory (Angelucci et al., 2002).

5.2.3. Sleep deprivation and caffeine

Our findings revealed that a low dose of chronic caffeine treatment had no significant effect on normal memory, but it prevented memory loss of recent information resulting from acute sleep deprivation. Thus, it seems that the low chronic dose of caffeine acted as a protector rather than a promoter of memory function. Interestingly, previous reports have demonstrated the neuroprotective effects of chronic intake of caffeine in animal models of ischemia (Sutherland et al., 1991) as well as in human subjects (Maia and de Mendonca, 2002). Furthermore, the beneficial effect of caffeine on learning and memory is supported by an epidemiological study, which indicates an inverse correlation between coffee intake and the incidence of Alzheimer's

disease later in life (Maia and de Mendonca, 2002). Consistent with this view, a large body of evidence has shown that caffeine administration may be a protective factor against memory impairment resulting from a variety of animal models of brain disorders including Alzheimer's disease (Arendash et al., 2006; Dall'Igna et al., 2007; Arendash et al., 2009; Cao et al., 2009), Parkinson's disease (Gevaerd et al., 2001), attention deficit hyperactivity disorder (Prediger et al., 2005), age-related cognitive decline (Riedel and Jolles, 1996; Costa et al., 2008a), scopolamine-induced amnesia (Riedel et al., 1995), and epilepsy (Cognato et al., 2010).

It is worth mentioning that caffeine as a stimulant may have influenced sleep pattern on the pedestals by lightening sleep, thus reducing the incidents of falling into the water. As a stimulant, caffeine may also improve the behavioral performance in the RAWM in sleep-deprived rats, thus contributing to the enhanced performance compared to untreated sleep deprivation rats. However, the finding that caffeine prevents the sleep deprivation-induced LTP deficit and changes in molecular levels of signaling molecules for memory and synaptic plasticity in the hippocampus may be difficult to explain as consequences of the stimulant action of caffeine.

5.3. Electrophysiological experiments

Information storage is dependent upon changes in synaptic efficacy that strengthen the connection between two neurons. Long-term potentiation is generally

considered the closest cellular model for storing new information within neuronal networks (Malenka and Bear, 2004). Indeed, the expression properties of LTP in an animal correlate with the characteristics of learning and memory. Consistent with this picture, accumulated evidence demonstrates that LTP as well as memory are impaired during aging (Rosenzweig and Barnes, 2003; Shukitt-Hale et al., 2004), chronic stress (Gerges et al., 2001; Diamond et al., 2004; Gerges et al., 2004b), AD (Chapman et al., 1999; Srivareerat et al., 2008), epilepsy (Kleschevnikov et al., 1994), and many other cases.

5.3.1 Sleep deprivation and long-term potentiation

A substantial body of evidence confirms the detrimental impact of sleep deprivation on hippocampus-dependent LTP. Consistent with earlier findings, the results of the current electrophysiological investigations show that sleep deprivation causes impairment of hippocampal E-LTP and L-LTP in the pyramidal neurons of area CA1 as well as in the granule cells of the DG area of anaesthetized rats. Impairment of E-LTP and L-LTP as a result of sleep loss has been reported *in vivo* as well as *in vitro* (McDermott et al., 2003; Kim et al., 2005; Marks and Wayner, 2005; McDermott et al., 2006).

Impairment of synaptic plasticity in the hippocampus may be due to an imbalance between calcineurin and P-CaMKII. Previous reports from this lab showed

that, in various brain disorders that impaired synaptic plasticity, the levels of P-CaMKII during expression of E-LTP were also significantly impaired compared to that of control (Gerges et al., 2005; Aleisa et al., 2006c; Srivareerat et al., 2008). In the present report, sleep deprivation may have caused E-LTP deficit by preventing the increase in the levels of P-CaMKII normally seen after HFS. In addition, our experiments indicate a significant decrease in the basal levels of CaMKII (phosphorylated and total) associated with 24 hr of sleep loss, which could be as a result of decreased gene expression of CaMKII in the hippocampus as reported in the 8 hr and 48 hr sleep deprivation experiments (Guzman-Marin et al., 2006).

The principal layers of the CA1 and DG regions are involved in the flow of information within the hippocampal formation (Amaral and Witter, 1989; Gutierrez and Heinemann, 1999). Although there is extensive interaction between area CA1 and DG that contributes to the intrinsic flow of information within the hippocampus, the DG granule cells appear to be more resistant to several conditions including stress, anoxia, transient cerebral ischemia, obesity, and hypothyroidism than area CA1 pyramidal cells (Hsu et al., 1998; Yao et al., 1998; Gerges et al., 2001; Gerges and Alkadhi, 2004; Alzoubi et al., 2005b). In addition, granule cells have been reported to have stronger ability to express more BDNF mRNA than CA1 pyramidal neurons (Song et al., 2001). Furthermore, after transient cerebral ischemia, the functionality and the expression of

NMDA receptors decrease in area CA1 but not in the DG region. Indeed, ischemia decreases NMDA receptor mediated slow f-EPSP in addition to non-NMDA receptor mediated fast f-EPSP in the CA1 area but not in the DG area (Hsu et al., 1998). Therefore, the finding that the DG is as severely impaired by sleep loss as area CA1 suggests the particularly powerful impact of sleep loss.

The environmental stress in the pedestal method is often cited as a drawback that interferes with studying sleep deprivation. We have consistently shown that six weeks of psychosocial stress impairs hippocampal-dependent LTP in area CA1 but not in the DG area (Gerges et al., 2001; Gerges et al., 2004b; Aleisa et al., 2006d). A major explanation for the intact LTP in the DG area of stressed rats was proposed to be curtailed de-phosphorylation process due to lower basal levels of calcineurin in the DG of stressed rats (Gerges et al., 2003b). Therefore, the finding that sleep deprivation causes LTP impairment in area CA1 as well as in the DG area (McDermott et al., 2003; Kim et al., 2005; Marks and Wayner, 2005) minimizes the role of stress in the effects on brain function of sleep deprivation in the modified multiple platform method.

Since electrophysiological experiments were conducted in animals under anesthesia, the possibility remains that the brain may be “sleeping” during anesthesia. Some anesthetics interfere with sleep; for example, both benzodiazepines and barbiturates are known to impact sleep patterns by interfering with the gamma amino-

butyric acid (GABA) receptor function (Curtis and Lodge, 1977; Maggi and Meli, 1986; Orser, 2006). In the current experiments, we used urethane, which acts by reducing the intrinsic excitability of neuronal membranes without affecting synaptic transmission (Maggi and Meli, 1986). This attribute made urethane suitable for the investigations of neural functions in the central nervous system.

5.3.2. Caffeine and LTP

Our results show that chronic use of caffeine in normal rats had no enhancement effect on either E-LTP or L-LTP in both CA1 and DG regions. However, a positive influence of caffeine on plastic changes in synaptic transmission has been reported. Indeed, it has been shown that caffeine induces an LTP-like response in the Schaffer collaterals-CA1 pyramidal neuron synapses in rat hippocampal slices (Martin and Buno, 2003). It is believed that caffeine enhances the excitability of rat hippocampal slices by antagonizing the effects of adenosine. In fact, it is well established, that in the hippocampus, endogenous adenosine interferes with synaptic plasticity through activation of the highly expressed adenosine A_1 receptors. Adenosine produces an inhibitory effect on LTP in area CA1 in rat hippocampal slices (de Mendonca and Ribeiro, 1994).

5.3.3. LTP impairment induced by acute sleep deprivation: effect of caffeine

On the cellular level, chronic intake of a low dose of caffeine protects the CA1 and DG regions against the negative effects of sleep deprivation. Interestingly, the present results show that the I/O relationship in the perforant path and Schaffer collateral synapses in the sleep deprivation group is not different than that of the control group, suggesting normal basal synaptic transmission in the sleep-deprived rats. Moreover, although chronic caffeine administration markedly inhibits the harmful effect of sleep deprivation on E-LTP and L-LTP, it does not appear to improve the basal synaptic transmission.

Results of the current study show that chronic caffeine treatment partially prevented 24 hr sleep deprivation-induced long-term memory impairment whereas it completely prevented impairment of L-LTP induced by the same period of sleep loss. The reason for the variable effect of caffeine on long-term memory compared to that on L-LTP in sleep-deprived rats is unknown. However, it can be related to the sensitivity of the tests. It is also possible that areas other than the CA1 or DG areas, which are involved in long-term memory, are affected by acute sleep deprivation.

5.4. Molecular experiments:

5.4.1 CaMKII

The significance of CaMKII in the induction of LTP is reflected in the findings that active CaMKII produces LTP-like synaptic potentiation in hippocampal slices (Pettit et al., 1994). Furthermore, mutant mice lacking CaMKII show impairment in spatial memory and LTP (Silva et al., 1992b; Silva et al., 1992a). In this study, we found that sleep deprivation reduces the levels of total-CaMKII and P-CaMKII in CA1 and DG areas. At the cellular level, the beneficial effect of caffeine in sleep-deprived rats may be attributed to its ability to prevent the decrease in the basal levels of P-CaMKII. The unchanged ratio of basal P-CaMKII/ total-CaMKII in the sleep-deprived rats suggests an overall decrease in the total protein levels of CaMKII. Thus, maintaining the basal levels of t-CaMKII by caffeine during sleep deprivation is probably responsible for maintaining the production of adequate P-CaMKII levels, which positively impacts short-term memory as well as E-LTP expression in CA1 and DG areas.

The protein levels of total-CaMKII, when measured during expression of LTP, were increased in all stimulated groups, including the sleep deprivation group compared to the un-stimulated control group. However, the levels of P-CaMKII increased in the stimulated control and caffeine groups but not in the stimulated sleep deprivation group, in which LTP was also reduced. The failure of repetitive stimulation to increase P-

CaMKII levels in the sleep deprivation group was prevented by chronic intake of caffeine. The electrophysiological and molecular results stress the essential function of P-CaMKII in the expression of LTP, which is in agreement with previous findings (Malenka et al., 1989; Pettit et al., 1994; Thomas et al., 1994; Lledo et al., 1995).

5.4.2 Calcineurin

Calcineurin, a phosphatase enzyme, is an essential signaling molecule for the regulation of memory and synaptic plasticity through dephosphorylating P-CaMKII. Evidence indicates that calcineurin reduces post-synaptic activity and impairs LTP in the hippocampus (Wang and Kelly, 1997; Winder et al., 1998). It has been reported that over-expression of calcineurin in hippocampus impairs LTP (Winder et al., 1998) and that pharmacological inhibitors of calcineurin facilitate LTP in hippocampal slices (Wang and Kelly, 1997). Calcineurin exerts its action by suppressing natural inhibitor-1, which leads to stimulation of protein phosphatase 1 (PP1) (Mulkey et al., 1994). The activated PP1 dephosphorylates P-CaMKII, which results in reduction of memory retention (Wang and Kelly, 1997).

In agreement with earlier findings, which reported that 72 hr of sleep loss does not affect basal levels of calcineurin in the hippocampus (Wang et al., 2009), the present findings indicated that protein levels of calcineurin in the total homogenate of the CA1 and DG areas were not affected by 24 hr of sleep deprivation. Furthermore, it has been

reported that sleep deprivation does not influence the level of PP1, which is activated by calcineurin (Guan et al., 2004). Although our results showed that sleep deprivation does not change the basal levels of calcineurin in the CA1 and DG areas of the hippocampus, the possibility remains that sleep deprivation may affect calcineurin activity only or the level/activity of another phosphatase. In fact, the activity of calcineurin has been reported to increase after 72 hr of sleep deprivation (Wang et al., 2009).

The levels of calcineurin, measured during expression of LTP, were increased markedly in all stimulated groups, including the sleep deprivation group, compared to the un-stimulated control group, which is consistent with previous findings from this laboratory (Alzoubi et al., 2005a; Aleisa et al., 2006c). The increase in the calcineurin level may work to reduce excessive activation of hippocampal neurons by normalizing the level of kinases thus allowing the process of learning to continue or to prevent saturation of LTP and permit the induction of another LTP.

5.4.3. BDNF

The protein family of neurotrophins, including BDNF, is well known to regulate the survival of neurons and encourage growth and differentiation of new neurons and synapses (Lewin and Barde, 1996; Huang and Reichardt, 2001). In the last decades, accumulated evidence suggests that BDNF plays an additional pivotal role in

hippocampal synaptic plasticity and spatial learning (Lessmann et al., 1994; Kang and Schuman, 1995; Figurov et al., 1996; Kesslak et al., 1998). BDNF influences synaptic plasticity by raising the efficiency of synaptic transmission through activation of CaMKII and CREB in the hippocampus (Finkbeiner et al., 1997; Boulanger and Poo, 1999). In the hippocampus, BDNF enhances activation of CaMKII and CREB, probably by a mechanism that involves the release of Ca^{2+} from the internal stores through tyrosine kinase B (TrkB) receptor/phospholipase Cγ (PLCγ) pathway (Blanquet and Lamour, 1997; Finkbeiner et al., 1997; Minichiello et al., 2002). Brain-derived neurotrophic factor binds to TrkB receptors and activates the PLCγ signaling pathway to generate diacylglycerol (DAG) and inositol tri-phosphate (IP_3), and the latter releases Ca^{2+} from cytoplasmic stores.

Acute sleep deprivation reduced the basal levels of BDNF in the CA1 and DG areas which is consistent with the finding that 8 and 48 hr periods of sleep deprivation decrease the gene expression and the protein levels of BDNF in the hippocampus (Guzman-Marin et al., 2006). The decreased basal levels of BDNF in sleep-deprived rats shown in the current study may be considered a factor involved in the reduction of both P-CaMKII and P-CREB basal levels. It appears that by preventing the reduction in the basal levels of BDNF, caffeine prevents the sleep deprivation-induced reduction in the

levels of P-CaMKII and P-CREB and subsequent impairment of E-LTP and L-LTP, respectively in the CA1 and DG regions.

Brain-derived neurotrophic factor is activity dependent (Mowla et al., 1999; Farhadi et al., 2000; Lu, 2003) and can be induced by HFS input to the hippocampus (Balkowiec and Katz, 2000; Hartmann et al., 2001; Gartner and Staiger, 2002). In this study, we did not detect alterations in the protein levels of BDNF measured one hr after HFS in all stimulated groups compared to those of un-stimulated control group in CA1 and DG regions. Our finding supports earlier reports postulating that BDNF plays a transient function for a limited time during the induction of synaptic potentiation but not during LTP expression in the hippocampus (Kossel et al., 2001).

Five hrs after MHFS, the protein levels of BDNF were increased in the CA1 and DG areas of the stimulated control and caffeine groups but not in the stimulated sleep deprivation group. Chronic caffeine treatment prevented the sleep deprivation-induced decrease in BDNF protein levels. It has been revealed that BDNF activates CREB-dependent protein synthesis, and that the BDNF gene is considered to be one of the CREB targets (Patterson et al., 1992; Finkbeiner et al., 1997; Shieh et al., 1998; Tao et al., 1998). As a consequence, BDNF synthesis can be stimulated upon CREB activation. Our findings demonstrated normal activation of CREB in caffeine and caffeine-treated sleep-deprived rats. It appeared that the levels of BDNF during the expression of L-LTP follow

those of P-CREB. Furthermore, it has been found that applying BDNF after stimulation is adequate to express L-LTP in slices treated with a protein synthesis inhibitor (Pang and Lu, 2004; Pang et al., 2004). Therefore, the inability of MHFS to enhance the BDNF levels in sleep-deprived rats and the significantly higher levels of BDNF in stimulated caffeine treated sleep-deprived rats could be responsible for the positive effect of caffeine against L-LTP deficit induced by sleep deprivation.

5.4.4. cAMP response element binding protein (CREB)

CREB mediates the synthesis of proteins that are important for long-term synaptic plasticity and memory. The gene expression of CREB has been found to decrease after 8 and 48 hr periods of sleep loss in the hippocampus (Guzman-Marin et al., 2006). This reduction could be attributed to the significant decrease in the upstream modulators of CREB including extracellular signal-regulated kinase (ERK) and calcium/calmodulin-dependent protein kinase IV (CaMKIV). In fact, it has been reported that sleep loss decreases the levels of ERK phosphorylation (Guan et al., 2004). In the present study, we found that acute sleep deprivation decreased the basal protein levels of total-CREB and P-CREB in CA1 area as well as in DG area. Additionally, chronic low dose of caffeine consumption prevented sleep deprivation-induced decrease in the levels of total-CREB and P-CREB in the hippocampus without affecting their levels in normal rats.

After expression of L-LTP, the levels of total-CREB were increased in all stimulated groups. However, the levels of P-CREB were increased in the stimulated control, caffeine, and caffeine treated sleep deprivation groups, but not the stimulated sleep deprivation group. The inability of MHFS to increase the phosphorylation of CREB could be responsible for the impairment of L-LTP in the CA1 and DG areas of sleep-deprived rats. Furthermore, the ability of caffeine to prevent the decrease in P-CREB levels after MHFS emphasizes the role of P-CREB in the L-LTP decline associated with 24 hr sleep deprivation.

5.4.5. CaMKIV

Mice with mutation in CaMKIV show impairment in L-LTP, long-term memory and CREB phosphorylation in the hippocampus (Ho et al., 2000; Kang et al., 2001). However, these mutant mice have normal E-LTP and short-term memory. Our results revealed reduced basal protein levels of CaMKIV in CA1 and DG areas of the hippocampus in the sleep deprivation group. This decrease was prevented by chronic caffeine treatment of sleep-deprived rats. It seems that reduced CaMKIV levels is a factor in the reduced levels of CREB phosphorylation in sleep-deprived rats leading to impairment of L-LTP. Conversely, the normal levels of CaMKIV in caffeine treated sleep deprived rats could contribute to normal CREB phosphorylation and intact L-LTP seen in the caffeine/sleep deprivation group.

After MHFS, the levels of CaMKIV were increased in all stimulated groups. The increase in the levels of CaMKIV was also observed after using a theta burst stimulation protocol in area CA1 (Tokuda et al., 1997). This enhancement supports the role of CaMKIV in the expression of L-LTP.

5.5. How does caffeine protect against sleep deprivation-induced memory impairment in hippocampus?

Considerable evidence shows that adenosine accumulates during sustained wakefulness to suppress neuronal activity and to promote sleep (Porkka-Heiskanen, 1999). In the hippocampus, adenosine A_1 receptors are expressed at higher levels than A_{2a} receptors and are considered the predominant adenosine receptors. Adenosine A_1 receptors are coupled to inhibitory G-proteins which decrease the levels of cyclic adenosine mono-phosphate (cAMP). Under normal conditions, adenosine preferentially activates A_1 receptors to inhibit neurotransmitter release. A growing body of evidence suggests that endogenous adenosine interferes with synaptic plasticity through activation of the highly expressed adenosine A_1 receptors in the hippocampus. Adenosine produces an inhibitory effect on LTP in rat hippocampal slices and disrupts the process of learning and memory at the synaptic level (de Mendonca and Ribeiro, 1994). Additionally, adenosine inhibits the release of glutamate from nerve terminals, and at post-synaptic membrane; it stabilizes the magnesium ion on NMDA receptors,

therefore prevents NMDA receptor mediated excitation (de Mendonca et al., 1995; Dunwiddie and Masino, 2001). Overall, the increase in the levels of adenosine during sleep deprivation could adversely affect hippocampus-dependent learning and memory through its action on A_1 receptors.

The protective mechanism of chronic caffeine treatment against sleep deprivation-induced E-LTP and L-LTP impairment may involve preventing the decrease in the levels of P-CaMKII and P-CREB, respectively, in sleep-deprived rats (fig. 36). However, the precise mechanism by which chronic caffeine treatment prevents the effects of sleep deprivation on memory and LTP is not clearly understood. Although caffeine can act through several mechanisms including antagonism of adenosine receptors (Nehlig et al., 1992), phosphodiesterase enzyme inhibition (Smellie et al., 1979), and increasing calcium induced-calcium release (McPherson et al., 1991), it has been suggested that the concentration of caffeine typically consumed by humans, which is comparable to the caffeine dose used in the current project, may act mainly by inhibiting adenosine receptors (Fredholm, 1995). By antagonizing the abundant A_1 receptors, caffeine disrupts the signaling cascades mediated by adenosine on the pre-synaptic neurons as well as on the post-synaptic neurons.

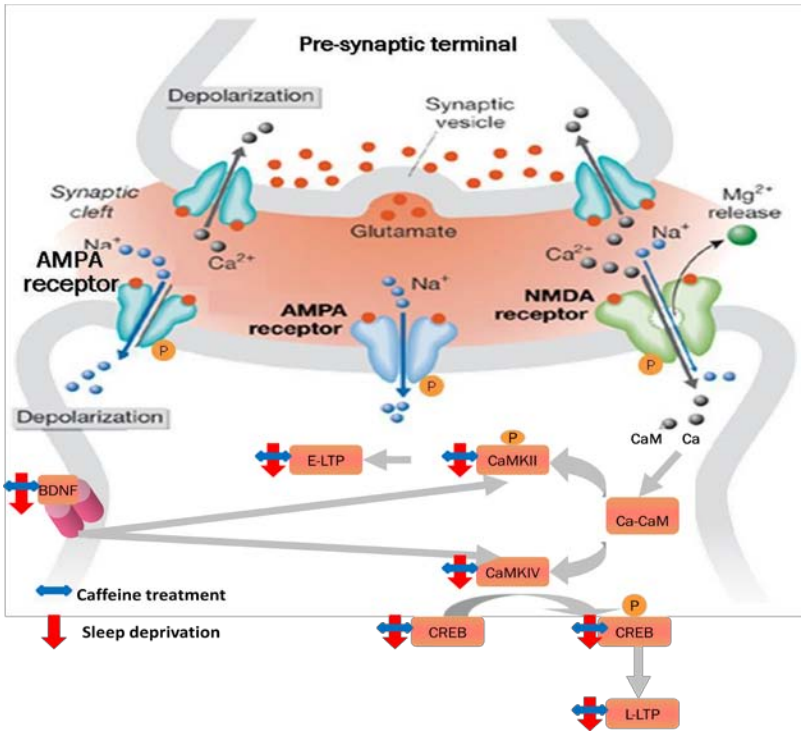


Figure 36: The sequence of events in the chronic caffeine treatment on the sleep deprivation-induced changes of the key signaling molecules responsible for the expression of E-LTP and L-LTP. The scheme shows the effect of sleep deprivation (red arrow) on the basal levels of CaMKII and CREB, which mediate E-LTP and L-LTP, respectively. Chronic caffeine treatment before sleep deprivation (blue arrow) protects against the changes in the CaMKII and CREB in the sleep-deprived rats, resulting in normal E-LTP and L-LTP in CA1 and DG regions of the hippocampus (modified from Voglis and Tavernarakis, 2006).

6. SUMMARY AND CONCLUSIONS

1. Under our experimental conditions, chronic caffeine treatment alone has no effect on learning and memory in normal animals. However, in the presence of sleep deprivation, caffeine antagonizes the deleterious effect of sleep deprivation on learning, short-term memory, and long-term memory. These results strengthen our view that caffeine acts only in a need-dependent manner (i.e., when there is memory impairment). Therefore, caffeine seems to be a protector rather than a promoter of memory function.

2. On the cellular level, our results show that caffeine treatment for 4 weeks protects against sleep deprivation-induced E-LTP impairment in the CA1 and DG areas. The failure of HFS to produce LTP in the sleep-deprived rats may be explained by the findings that, during expression of LTP, the stimulated protein levels of P-CaMKII do not increase compared to the other three experimental groups in the total homogenate of CA1 and DG areas. Therefore, we propose that the beneficial effect of caffeine at the cellular level may be attributed to its ability to prevent the decrease in P-CaMKII induced by sleep deprivation during expression of E-LTP.

3. Chronic caffeine treatment prevents sleep deprivation-induced reduction of the basal levels of CaMKII (total and phosphorylated), and BDNF. The reduction in the basal levels of P-CaMKII may be due to sleep deprivation-induced reduction in total CaMKII and BDNF basal levels.

4. The present findings indicate that protein levels of calcineurin in the total homogenate of the CA1 and DG areas are not affected by 24 hr of sleep deprivation. However, the possibility remains that sleep deprivation may affect calcineurin activity without affecting its levels, or it may act by affecting the level/activity of different phosphatase.

5. MHFS that evoked L-LTP produces a significant increase in the levels of total CREB, and CaMKIV in the CA1 and DG regions of all stimulated groups. While MHFS increases the levels of P-CREB and BDNF, which are important for the expression of L- LTP in the CA1 and DG regions of control or caffeine rats, it fails to increase the levels of these molecules in sleep-deprived rats, thus suggesting a reduction of signaling molecule levels as a possible mechanism by which sleep deprivation impairs the expression of L-LTP. Treating sleep-deprived rats chronically with caffeine enables MHFS to evoke L-LTP and increases the levels of P-CREB.

6. Sleep deprivation reduces the basal levels of P-CREB in CA1 and DG areas of the hippocampus. This reduction in the basal levels of P-CREB could be due to sleep deprivation-induced reduction in total-CREB, and reduction of upstream modulators such as CaMKIV and P-MAPKp44/42. Chronic caffeine treatment normalizes the basal levels of CREB (phosphorylated and total), and the levels of upstream modulator of CREB (CaMKIV) during sleep deprivation, which probably led to normal long-term memory as well as expression of normal L-LTP in caffeine-treated sleep-deprived rats.

7. All in all, the current study provides the knowledge for the cellular and molecular mechanisms of how caffeine, as self medication, may protect against spatial memory impairment associated with acute sleep deprivation.

7. REFERENCES:

Abel T, Kandel E (1998) Positive and negative regulatory mechanisms that mediate long-term memory storage. Brain Res Brain Res Rev 26:360-378.

Abel T, Martin KC, Bartsch D, Kandel ER (1998) Memory suppressor genes: inhibitory constraints on the storage of long-term memory. Science 279:338-341.

Akaneya Y, Tsumoto T, Kinoshita S, Hatanaka H (1997) Brain-derived neurotrophic factor enhances long-term potentiation in rat visual cortex. J Neurosci 17:6707-6716.

Alamed J, Wilcock DM, Diamond DM, Gordon MN, Morgan D (2006) Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice. Nat Protoc 1:1671-1679.

Alarcon JM, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, Barco A (2004) Chromatin acetylation, memory, and LTP are impaired in CBP+/- mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron 42:947-959.

Aleisa AM, Alzoubi KH, Alkadhi KA (2006a) Chronic but not acute nicotine treatment reverses stress-induced impairment of LTP in anesthetized rats. Brain Res 1097:78-84.

Aleisa AM, Alzoubi KH, Alkadhi KA (2006b) Nicotine prevents stress-induced enhancement of long-term depression in hippocampal area CA1: electrophysiological and molecular studies. J Neurosci Res 83:309-317.

Aleisa AM, Alzoubi KH, Gerges NZ, Alkadhi KA (2006c) Chronic psychosocial stress-induced impairment of hippocampal LTP: possible role of BDNF. Neurobiol Dis 22:453-462.

Aleisa AM, Alzoubi KH, Gerges NZ, Alkadhi KA (2006d) Nicotine blocks stress-induced impairment of spatial memory and long-term potentiation of the hippocampal CA1 region. Int J Neuropsychopharmacol 9:417-426.

Alonso M, Vianna MR, Depino AM, Mello e Souza T, Pereira P, Szapiro G, Viola H, Pitossi F, Izquierdo I, Medina JH (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus 12:551-560.

Alzoubi KH, Alkadhi KA (2007) A critical role of CREB in the impairment of late-phase LTP by adult onset hypothyroidism. Exp Neurol 203:63-71.

Alzoubi KH, Gerges NZ, Alkadhi KA (2005a) Levothyroxin restores hypothyroidism-induced impairment of LTP of hippocampal CA1: electrophysiological and molecular studies. Exp Neurol 195:330-341.

Alzoubi KH, Aleisa AM, Alkadhi KA (2005b) Impairment of long-term potentiation in the CA1, but not dentate gyrus, of the hippocampus in Obese Zucker rats: role of calcineurin and phosphorylated CaMKII. J Mol Neurosci 27:337-346.

Alzoubi KH, Aleisa AM, Alkadhi KA (2006) Molecular studies on the protective effect of nicotine in adult-onset hypothyroidism-induced impairment of long-term potentiation. Hippocampus 16:861-874.

Alzoubi KH, Aleisa AM, Alkadhi KA (2007a) Nicotine prevents disruption of the late phase LTP-related molecular cascade in adult-onset hypothyroidism. Hippocampus 17:654-664.

Alzoubi KH, Aleisa AM, Alkadhi KA (2007b) Adult-onset hypothyroidism facilitates and enhances LTD: reversal by chronic nicotine treatment. Neurobiol Dis 26:264-272.

Alzoubi KH, Gerges NZ, Aleisa AM, Alkadhi KA (2009a) Levothyroxin restores hypothyroidism-induced impairment of hippocampus-dependent learning and memory: Behavioral, electrophysiological, and molecular studies. Hippocampus 19:66-78.

Alzoubi KH, Abdul-Razzak KK, Khabour OF, Al-Tuweiq GM, Alzubi MA, Alkadhi KA (2009b) Adverse effect of combination of chronic psychosocial stress and high fat diet on hippocampus-dependent memory in rats. Behav Brain Res 204:117-123.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31:571-591.

Angelucci ME, Cesario C, Hiroi RH, Rosalen PL, Da Cunha C (2002) Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res 35:1201-1208.

Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, Cracchiolo JR, Shippy D, Tan J (2006) Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. Neuroscience 142:941-952.

Arendash GW, Mori T, Cao C, Mamcarz M, Runfeldt M, Dickson A, Rezai-Zadeh K, Tane J, Citron BA, Lin X, Echeverria V, Potter H (2009) Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. J Alzheimers Dis 17:661-680.

Ascher P, Nowak L (1988) The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. J Physiol 399:247-266.

Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neurol 50:56-63.

Astur RS, Taylor LB, Mamelak AN, Philpott L, Sutherland RJ (2002) Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. Behav Brain Res 132:77-84.

Bailey CH, Bartsch D, Kandel ER (1996) Toward a molecular definition of long-term memory storage. Proc Natl Acad Sci U S A 93:13445-13452.

Balkowiec A, Katz DM (2000) Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. J Neurosci 20:7417-7423.

Barco A, Alarcon JM, Kandel ER (2002) Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell 108:689-703.

Barco A, Bailey CH, Kandel ER (2006) Common molecular mechanisms in explicit and implicit memory. J Neurochem 97:1520-1533.

Barco A, Patterson S, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER (2005) Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48:123-137.

Barria A, Derkach V, Soderling T (1997a) Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. J Biol Chem 272:32727-32730.

Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997b) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276:2042-2045.

Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH (2008) BDNF is essential to promote persistence of long-term memory storage. Proc Natl Acad Sci U S A 105:2711-2716.

Bito H, Deisseroth K, Tsien RW (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. Cell 87:1203-1214.

Bjorness TE, Riley BT, Tysor MK, Poe GR (2005) REM restriction persistently alters strategy used to solve a spatial task. Learn Mem 12:352-359.

Blanquet PR, Lamour Y (1997) Brain-derived neurotrophic factor increases Ca2+/calmodulin-dependent protein kinase 2 activity in hippocampus. J Biol Chem 272:24133-24136.

Blissitt PA (2001) Sleep, memory, and learning. J Neurosci Nurs 33:208-215.

Bolshakov VY, Golan H, Kandel ER, Siegelbaum SA (1997) Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. Neuron 19:635-651.

Boulanger L, Poo MM (1999) Gating of BDNF-induced synaptic potentiation by cAMP. Science 284:1982-1984.

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

Buhot MC, Wolff M, Benhassine N, Costet P, Hen R, Segu L (2003) Spatial learning in the 5-HT1B receptor knockout mouse: selective facilitation/impairment depending on the cognitive demand. Learn Mem 10:466-477.

Buresova O, Bures J, Oitzl MS, Zahalka A (1985) Radial maze in the water tank: an aversively motivated spatial working memory task. Physiol Behav 34:1003-1005.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35:625-641.

Cai DJ, Shuman T, Gorman MR, Sage JR, Anagnostaras SG (2009) Sleep selectively enhances hippocampus-dependent memory in mice. Behav Neurosci 123:713-719.

Campbell IG, Guinan MJ, Horowitz JM (2002) Sleep deprivation impairs long-term potentiation in rat hippocampal slices. J Neurophysiol 88:1073-1076.

Cao C, Cirrito JR, Lin X, Wang L, Verges DK, Dickson A, Mamcarz M, Zhang C, Mori T, Arendash GW, Holtzman DM, Potter H (2009) Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. J Alzheimers Dis 17:681-697.

Capek S, Guenther RK (2009) Caffeine's effects on true and false memory. Psychol Rep 104:787-795.

Castren E, Pitkanen M, Sirvio J, Parsadanian A, Lindholm D, Thoenen H, Riekkinen PJ (1993) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport 4:895-898.

Cestari V, Castellano C (1996) Caffeine and cocaine interaction on memory consolidation in mice. Arch Int Pharmacodyn Ther 331:94-104.

Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TV, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat Neurosci 2:271-276.

Chen C, Hardy M, Zhang J, LaHoste GJ, Bazan NG (2006) Altered NMDA receptor trafficking contributes to sleep deprivation-induced hippocampal synaptic and cognitive impairments. Biochem Biophys Res Commun 340:435-440.

Cognato GP, Agostinho PM, Hockemeyer J, Muller CE, Souza DO, Cunha RA (2010) Caffeine and an adenosine A(2A) receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. J Neurochem 112:453-462.

Cohen NJ, Squire LR (1980) Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. Science 210:207-210.

Colombo PJ, Brightwell JJ, Countryman RA (2003) Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. J Neurosci 23:3547-3554.

Costa MS, Botton PH, Mioranzza S, Souza DO, Porciuncula LO (2008a) Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tirosine kinase receptor (TrkB) content in mice. Neuroscience 153:1071-1078.

Costa MS, Botton PH, Mioranzza S, Ardais AP, Moreira JD, Souza DO, Porciuncula LO (2008b) Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus. Neurochem Int 53:89-94.

Curtis DR, Lodge D (1977) Pentobarbitone enhancement of the inhibitory action of GABA. Nature 270:543-544.

Dall'Igna OP, Souza DO, Lara DR (2004) Caffeine as a neuroprotective adenosine receptor antagonist. Ann Pharmacother 38:717-718.

Dall'Igna OP, Fett P, Gomes MW, Souza DO, Cunha RA, Lara DR (2007) Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25-35)-induced cognitive deficits in mice. Exp Neurol 203:241-245.

Dash PK, Hochner B, Kandel ER (1990) Injection of the cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. Nature 345:718-721.

Datta S, Mavanji V, Ulloor J, Patterson EH (2004) Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: a mechanism for sleep-dependent plasticity. J Neurosci 24:1416-1427.

de Mendonca A, Ribeiro JA (1994) Endogenous adenosine modulates long-term potentiation in the hippocampus. Neuroscience 62:385-390.

de Mendonca A, Sebastiao AM, Ribeiro JA (1995) Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurones by adenosine A1 receptor activation. Neuroreport 6:1097-1100.

Devan BD, Goad EH, Petri HL (1996) Dissociation of hippocampal and striatal contributions to spatial navigation in the water maze. Neurobiol Learn Mem 66:305-323.

Diamond DM, Park CR, Woodson JC (2004) Stress generates emotional memories and retrograde amnesia by inducing an endogenous form of hippocampal LTP. Hippocampus 14:281-291.

Diamond DM, Park CR, Heman KL, Rose GM (1999) Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus 9:542-552.

Diekelmann S, Born J (2010) The memory function of sleep. Nat Rev Neurosci 11:114-126.

Dixon AK, Gubitz AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC (1996) Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol 118:1461-1468.

Doyere V, Errington ML, Laroche S, Bliss TV (1996) Low-frequency trains of paired stimuli induce long-term depression in area CA1 but not in dentate gyrus of the intact rat. Hippocampus 6:52-57.

Dragunow M, Beilharz E, Mason B, Lawlor P, Abraham W, Gluckman P (1993) Brain-derived neurotrophic factor expression after long-term potentiation. Neurosci Lett 160:232-236.

Dubiela FP, de Oliveira MG, Moreira KD, Nobrega JN, Tufik S, Hipolide DC (2010) Inverse benzodiazepine agonist beta-CCM does not reverse learning deficit induced by sleep deprivation. Neurosci Lett 469:169-173.

Dubreuil D, Tixier C, Dutrieux G, Edeline JM (2003) Does the radial arm maze necessarily test spatial memory? Neurobiol Learn Mem 79:109-117.

Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24:31-55.

Durlach PJ (1998) The effects of a low dose of caffeine on cognitive performance. Psychopharmacology (Berl) 140:116-119.

Farhadi HF, Mowla SJ, Petrecca K, Morris SJ, Seidah NG, Murphy RA (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. J Neurosci 20:4059-4068.

Ferrara M, Iaria G, Tempesta D, Curcio G, Moroni F, Marzano C, De Gennaro L, Pacitti C (2008) Sleep to find your way: the role of sleep in the consolidation of memory for navigation in humans. Hippocampus 18:844-851.

Ferre S (2008) An update on the mechanisms of the psychostimulant effects of caffeine. J Neurochem 105:1067-1079.

Ferre S, Fredholm BB, Morelli M, Popoli P, Fuxe K (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 20:482-487.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 381:706-709.

Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM, Reppert SM (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Brain Res Mol Brain Res 14:186-195.

Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19:1031-1047.

Fisher S, Guillet R (1997) Neonatal caffeine alters passive avoidance retention in rats in an age- and gender-related manner. Brain Res Dev Brain Res 98:145-149.

Foskett A, Ali A, Gant N (2009) Caffeine enhances cognitive function and skill performance during simulated soccer activity. Int J Sport Nutr Exerc Metab 19:410-423.

Franken P, Tobler I, Borbely AA (1993) Effects of 12-h sleep deprivation and of 12-h cold exposure on sleep regulation and cortical temperature in the rat. Physiol Behav 54:885-894.

Fredholm BB (1995) Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol Toxicol 76:93-101.

Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 51:83-133.

Fujii S, Saito K, Miyakawa H, Ito K, Kato H (1991) Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. Brain Res 555:112-122.

Fukunaga K, Miyamoto E (2000) A working model of CaM kinase II activity in hippocampal long-term potentiation and memory. Neurosci Res 38:3-17.

Fukunaga K, Muller D, Miyamoto E (1996) CaM kinase II in long-term potentiation. Neurochem Int 28:343-358.

Fukunaga K, Muller D, Ohmitsu M, Bako E, DePaoli-Roach AA, Miyamoto E (2000) Decreased protein phosphatase 2A activity in hippocampal long-term potentiation. J Neurochem 74:807-817.

Furusawa K (1991) Drug effects on cognitive function in mice determined by the non-matching to sample task using a 4-arm maze. Jpn J Pharmacol 56:483-493.

Gais S, Albouy G, Boly M, Dang-Vu TT, Darsaud A, Desseilles M, Rauchs G, Schabus M, Sterpenich V, Vandewalle G, Maquet P, Peigneux P (2007) Sleep transforms the cerebral trace of declarative memories. Proc Natl Acad Sci U S A 104:18778-18783.

Gartner A, Staiger V (2002) Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. Proc Natl Acad Sci U S A 99:6386-6391.

Gerges NZ, Alkadhi KA (2004) Hypothyroidism impairs late LTP in CA1 region but not in dentate gyrus of the intact rat hippocampus: MAPK involvement. Hippocampus 14:40-45.

Gerges NZ, Stringer JL, Alkadhi KA (2001) Combination of hypothyroidism and stress abolishes early LTP in the CA1 but not dentate gyrus of hippocampus of adult rats. Brain Res 922:250-260.

Gerges NZ, Aleisa AM, Alkadhi KA (2003a) Impaired long-term potentiation in obese zucker rats: possible involvement of presynaptic mechanism. Neuroscience 120:535-539.

Gerges NZ, Alzoubi KH, Alkadhi KA (2005) Role of phosphorylated CaMKII and calcineurin in the differential effect of hypothyroidism on LTP of CA1 and dentate gyrus. Hippocampus 15:480-490.

Gerges NZ, Aleisa AM, Schwarz LA, Alkadhi KA (2003b) Chronic psychosocial stress decreases calcineurin in the dentate gyrus: a possible mechanism for preservation of early ltp. Neuroscience 117:869-874.

Gerges NZ, Aleisa AM, Schwarz LA, Alkadhi KA (2004a) Reduced basal CaMKII levels in hippocampal CA1 region: possible cause of stress-induced impairment of LTP in chronically stressed rats. Hippocampus 14:402-410.

Gerges NZ, Alzoubi KH, Park CR, Diamond DM, Alkadhi KA (2004b) Adverse effect of the combination of hypothyroidism and chronic psychosocial stress on hippocampus-dependent memory in rats. Behav Brain Res 155:77-84.

Gevaerd MS, Takahashi RN, Silveira R, Da Cunha C (2001) Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Res Bull 55:101-106.

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870-873.

Glanzer M (1971) Short-term storage and long-term storage in recall. J Psychiatr Res 8:423-438.

Gohar A, Adams A, Gertner E, Sackett-Lundeen L, Heitz R, Engle R, Haus E, Bijwadia J (2009) Working memory capacity is decreased in sleep-deprived internal medicine residents. J Clin Sleep Med 5:191-197.

Gooney M, Shaw K, Kelly A, O'Mara SM, Lynch MA (2002) Long-term potentiation and spatial learning are associated with increased phosphorylation of TrkB and extracellular signal-regulated kinase (ERK) in the dentate gyrus: evidence for a role for brain-derived neurotrophic factor. Behav Neurosci 116:455-463.

Grahnstedt S, Ursin R (1985) Platform sleep deprivation affects deep slow wave sleep in addition to REM sleep. Behav Brain Res 18:233-239.

Graves LA, Heller EA, Pack AI, Abel T (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 10:168-176.

Guan Z, Peng X, Fang J (2004) Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res 1018:38-47.

Gutierrez R, Heinemann U (1999) Synaptic reorganization in explanted cultures of rat hippocampus. Brain Res 815:304-316.

Guzman-Marin R, Suntsova N, Stewart DR, Gong H, Szymusiak R, McGinty D (2003) Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. J Physiol 549:563-571.

Guzman-Marin R, Ying Z, Suntsova N, Methippara M, Bashir T, Szymusiak R, Gomez-Pinilla F, McGinty D (2006) Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. J Physiol 575:807-819.

Guzowski JF, McGaugh JL (1997) Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. Proc Natl Acad Sci U S A 94:2693-2698.

Hagewoud R, Havekes R, Novati A, Keijser JN, EA VDZ, Meerlo P (2009) Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. J Sleep Res.

Hameleers PA, Van Boxtel MP, Hogervorst E, Riedel WJ, Houx PJ, Buntinx F, Jolles J (2000) Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. Hum Psychopharmacol 15:573-581.

Han ME, Kim HJ, Lee YS, Kim DH, Choi JT, Pan CS, Yoon S, Baek SY, Kim BS, Kim JB, Oh SO (2009) Regulation of cerebrospinal fluid production by caffeine consumption. BMC Neurosci 10:110.

Harrison Y, Horne JA (2000) Sleep loss and temporal memory. Q J Exp Psychol A 53:271-279.

Hartmann M, Heumann R, Lessmann V (2001) Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. Embo J 20:5887-5897.

He J, Yamada K, Zou LB, Nabeshima T (2001) Spatial memory deficit and neurodegeneration induced by the direct injection of okadaic acid into the hippocampus in rats. J Neural Transm 108:1435-1443.

Heindel WC, Salmon DP, Shults CW, Walicke PA, Butters N (1989) Neuropsychological evidence for multiple implicit memory systems: a comparison of Alzheimer's, Huntington's, and Parkinson's disease patients. J Neurosci 9:582-587.

Herz RS (1999) Caffeine effects on mood and memory. Behav Res Ther 37:869-879.

Ho N, Liauw JA, Blaeser F, Wei F, Hanissian S, Muglia LM, Wozniak DF, Nardi A, Arvin KL, Holtzman DM, Linden DJ, Zhuo M, Muglia LJ, Chatila TA (2000) Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. J Neurosci 20:6459-6472.

Hodges H (1996) Maze procedures: the radial-arm and water maze compared. Brain Res Cogn Brain Res 3:167-181.

Howell LL, Coffin VL, Spealman RD (1997) Behavioral and physiological effects of xanthines in nonhuman primates. Psychopharmacology (Berl) 129:1-14.

Hsu JC, Zhang Y, Takagi N, Gurd JW, Wallace MC, Zhang L, Eubanks JH (1998) Decreased expression and functionality of NMDA receptor complexes persist in the CA1, but not in the dentate gyrus after transient cerebral ischemia. J Cereb Blood Flow Metab 18:768-775.

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677-736.

Hudzik TJ, Wenger GR (1993) Effects of drugs of abuse and cholinergic agents on delayed matching-to-sample responding in the squirrel monkey. J Pharmacol Exp Ther 265:120-127.

Ishikawa A, Kanayama Y, Matsumura H, Tsuchimochi H, Ishida Y, Nakamura S (2006) Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. Eur J Neurosci 24:243-248.

Jensen KF, Ohmstede CA, Fisher RS, Sahyoun N (1991a) Nuclear and axonal localization of Ca2+/calmodulin-dependent protein kinase type Gr in rat cerebellar cortex. Proc Natl Acad Sci U S A 88:2850-2853.

Jensen KF, Ohmstede CA, Fisher RS, Olin JK, Sahyoun N (1991b) Acquisition and loss of a neuronal Ca2+/calmodulin-dependent protein kinase during neuronal differentiation. Proc Natl Acad Sci U S A 88:4050-4053.

Kaang BK, Kandel ER, Grant SG (1993) Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. Neuron 10:427-435.

Kandel ER (2001a) The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 21:565-611.

Kandel ER, Schwartz JH (2001) Principles of neural science: Elsevier/North-Holland.

Kandel ERaS, J. H. (2001b) Principles of neural science.

Kang H, Schuman EM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658-1662.

Kang H, Sun LD, Atkins CM, Soderling TR, Wilson MA, Tonegawa S (2001) An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell 106:771-783.

Karnam HB, Zhou JL, Huang LT, Zhao Q, Shatskikh T, Holmes GL (2009) Early life seizures cause long-standing impairment of the hippocampal map. Exp Neurol 217:378-387.

Kerr JS, Sherwood N, Hindmarch I (1991) Separate and combined effects of the social drugs on psychomotor performance. Psychopharmacology (Berl) 104:113-119.

Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F (1998) Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? Behav Neurosci 112:1012-1019.

Kim EY, Mahmoud GS, Grover LM (2005) REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. Neurosci Lett 388:163-167.

Kiprianova I, Sandkuhler J, Schwab S, Hoyer S, Spranger M (1999) Brain-derived neurotrophic factor improves long-term potentiation and cognitive functions after transient forebrain ischemia in the rat. Exp Neurol 159:511-519.

Kleschevnikov AM, Sinden JD, Marchbanks R (1994) Fimbria-fornix lesions impair spatial performance and induce epileptic-like activity but do not affect long-term potentiation in the CA1 region of rat hippocampal slices. Brain Res 656:221-228.

Kopf SR, Melani A, Pedata F, Pepeu G (1999) Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. Psychopharmacology (Berl) 146:214-219.

Kopp C, Longordo F, Luthi A (2007) Experience-dependent changes in NMDA receptor composition at mature central synapses. Neuropharmacology 53:1-9.

Kopp C, Longordo F, Nicholson JR, Luthi A (2006) Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. J Neurosci 26:12456-12465.

Korkotian E, Segal M (1998) Fast confocal imaging of calcium released from stores in dendritic spines. Eur J Neurosci 10:2076-2084.

Korkotian E, Segal M (1999) Release of calcium from stores alters the morphology of dendritic spines in cultured hippocampal neurons. Proc Natl Acad Sci U S A 96:12068-12072.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 92:8856-8860.

Kossel AH, Cambridge SB, Wagner U, Bonhoeffer T (2001) A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. Proc Natl Acad Sci U S A 98:14702-14707.

Lessmann V, Gottmann K, Heumann R (1994) BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurones. Neuroreport 6:21-25.

Levitt RA (1966) Sleep deprivation in the rat. Science 153:85-87.

Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu Rev Neurosci 19:289-317.

Li S, Tian Y, Ding Y, Jin X, Yan C, Shen X (2009) The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. Learn Behav 37:246-253.

Lieberman HR, Wurtman RJ, Emde GG, Roberts C, Coviella IL (1987) The effects of low doses of caffeine on human performance and mood. Psychopharmacology (Berl) 92:308-312.

Linnarsson S, Bjorklund A, Ernfors P (1997) Learning deficit in BDNF mutant mice. Eur J Neurosci 9:2581-2587.

Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA (1995) Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. Proc Natl Acad Sci U S A 92:11175-11179.

Loke WH (1988) Effects of caffeine on mood and memory. Physiol Behav 44:367-372.

Lopes da Silva FH, Gorter JA, Wadman WJ (1986) Kindling of the hippocampus induces spatial memory deficits in the rat. Neurosci Lett 63:115-120.

Lu B (2003) BDNF and activity-dependent synaptic modulation. Learn Mem 10:86-98.

Lu KT, Wu SP, Gean PW (1999a) Promotion of forskolin-induced long-term potentiation of synaptic transmission by caffeine in area CA1 of the rat hippocampus. Chin J Physiol 42:249-253.

Lu YF, Kandel ER, Hawkins RD (1999b) Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. J Neurosci 19:10250-10261.

Lynch MA (2004) Long-term potentiation and memory. Physiol Rev 84:87-136.

Machado RB, Hipolide DC, Benedito-Silva AA, Tufik S (2004) Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. Brain Res 1004:45-51.

Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. Experientia 42:109-114.

Maia L, de Mendonca A (2002) Does caffeine intake protect from Alzheimer's disease? Eur J Neurol 9:377-382.

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

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.

Malenka RC, Kauer JA, Zucker RS, Nicoll RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81-84.

Malenka RC, Kauer JA, Perkel DJ, Mauk MD, Kelly PT, Nicoll RA, Waxham MN (1989) An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340:554-557.

Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245:862-866.

Mansuy IM, Winder DG, Moallem TM, Osman M, Mayford M, Hawkins RD, Kandel ER (1998) Inducible and reversible gene expression with the rtTA system for the study of memory. Neuron 21:257-265.

Marks CA, Wayner MJ (2005) Effects of sleep disruption on rat dentate granule cell LTP in vivo. Brain Res Bull 66:114-119.

Marshall L, Born J (2007) The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn Sci 11:442-450.

Martin ED, Buno W (2003) Caffeine-mediated presynaptic long-term potentiation in hippocampal CA1 pyramidal neurons. J Neurophysiol 89:3029-3038.

Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23:649-711.

McClelland JL, McNaughton BL, O'Reilly RC (1995) Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol Rev 102:419-457.

McDermott CM, Hardy MN, Bazan NG, Magee JC (2006) Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. J Physiol 570:553-565.

McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC (2003) Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 23:9687-9695.

McIntosh AR (2000) Towards a network theory of cognition. Neural Netw 13:861-870.

McNaughton BL, Barnes CA, Rao G, Baldwin J, Rasmussen M (1986) Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. J Neurosci 6:563-571.

McPherson PS, Kim YK, Valdivia H, Knudson CM, Takekura H, Franzini-Armstrong C, Coronado R, Campbell KP (1991) The brain ryanodine receptor: a caffeine-sensitive calcium release channel. Neuron 7:17-25.

Meissner WW (1966) Hippocampal functions in learning. J Psychiatr Res 4:235-304.

Messaoudi E, Bardsen K, Srebro B, Bramham CR (1998) Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. J Neurophysiol 79:496-499.

Miller G (2009) The brain collector. Science 324:1634-1636.

Minichiello L, Calella AM, Medina DL, Bonhoeffer T, Klein R, Korte M (2002) Mechanism of TrkB-mediated hippocampal long-term potentiation. Neuron 36:121-137.

Mirescu C, Peters JD, Noiman L, Gould E (2006) Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. Proc Natl Acad Sci U S A 103:19170-19175.

Miyamoto E, Fukunaga K (1996) A role of Ca2+/calmodulin-dependent protein kinase II in the induction of long-term potentiation in hippocampal CA1 area. Neurosci Res 24:117-122.

Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T (2000) Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci 20:7116-7121.

Modirrousta M, Mainville L, Jones BE (2005) Orexin and MCH neurons express c-Fos differently after sleep deprivation vs. recovery and bear different adrenergic receptors. Eur J Neurosci 21:2807-2816.

Mograss MA, Guillem F, Brazzini-Poisson V, Godbout R (2009) The effects of total sleep deprivation on recognition memory processes: a study of event-related potential. Neurobiol Learn Mem 91:343-352.

Morimoto K, Sato K, Sato S, Yamada N, Hayabara T (1998) Time-dependent changes in neurotrophic factor mRNA expression after kindling and long-term potentiation in rats. Brain Res Bull 45:599-605.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47-60.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683.

Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774-776.

Moser EI, Krobert KA, Moser MB, Morris RG (1998) Impaired spatial learning after saturation of long-term potentiation. Science 281:2038-2042.

Mowla SJ, Pareek S, Farhadi HF, Petrecca K, Fawcett JP, Seidah NG, Morris SJ, Sossin WS, Murphy RA (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. J Neurosci 19:2069-2080.

Mulkey RM, Herron CE, Malenka RC (1993) An essential role for protein phosphatases in hippocampal long-term depression. Science 261:1051-1055.

Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486-488.

National Sleep Foundation -Waking America to the importance of sleep {internet}. c. 2009. One-Third of Americans Lose Sleep Over Economy. 2009 March 2 {cited Feb 28 2010; {I screen}. Available from: http://www.sleepfoundation.org/article/press-release/one-third-americans-lose-sleep-over-economy.

Nayak AS, Moore CI, Browning MD (1996) Ca2+/calmodulin-dependent protein kinase II phosphorylation of the presynaptic protein synapsin I is persistently increased during long-term potentiation. Proc Natl Acad Sci U S A 93:15451-15456.

Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev 17:139-170.

Nguyen PV, Kandel ER (1996) A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. J Neurosci 16:3189-3198.

Nicholson AN, Stone BM (1980) Heterocyclic amphetamine derivatives and caffeine on sleep in man. Br J Clin Pharmacol 9:195-203.

O'Callaghan RM, Ohle R, Kelly AM (2007) The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. Behav Brain Res 176:362-366.

O'Dell TJ, Kandel ER (1994) Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. Learn Mem 1:129-139.

Ocampo-Garces A, Molina E, Rodriguez A, Vivaldi EA (2000) Homeostasis of REM sleep after total and selective sleep deprivation in the rat. J Neurophysiol 84:2699-2702.

Olton DS (1977) Spatial memory. Sci Am 236:82-84, 89-94, 96, 98.

Olton DS (1987) The radial arm maze as a tool in behavioral pharmacology. Physiol Behav 40:793-797.

Olton DS, Papas BC (1979) Spatial memory and hippocampal function. Neuropsychologia 17:669-682.

Orser BA (2006) Extrasynaptic GABAA receptors are critical targets for sedative-hypnotic drugs. J Clin Sleep Med 2:S12-18.

Ouyang Y, Kantor D, Harris KM, Schuman EM, Kennedy MB (1997) Visualization of the distribution of autophosphorylated calcium/calmodulin-dependent protein kinase II after tetanic stimulation in the CA1 area of the hippocampus. J Neurosci 17:5416-5427.

Packard MG, Hirsh R, White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. J Neurosci 9:1465-1472.

Pang PT, Lu B (2004) Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. Ageing Res Rev 3:407-430.

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487-491.

Papatheodoropoulos C, Kostopoulos G (2000) Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. Neurosci Lett 279:177-180.

Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M (1992) Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 9:1081-1088.

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16:1137-1145.

Peigneux P, Laureys S, Delbeuck X, Maquet P (2001) Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport 12:A111-124.

Pettit DL, Perlman S, Malinow R (1994) Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. Science 266:1881-1885.

Pires VA, Pamplona FA, Pandolfo P, Fernandes D, Prediger RD, Takahashi RN (2009) Adenosine receptor antagonists improve short-term object-recognition ability of spontaneously hypertensive rats: a rodent model of attention-deficit hyperactivity disorder. Behav Pharmacol 20:134-145.

Polzella DJ (1975a) Effects of sleep deprivation on short-term recognition memory. J Exp Psychol [Hum Learn] 104:194-200.

Polzella DJ (1975b) Effects of sleep deprivation on short-term recognition memory. J Exp Psychol Hum Learn 104:194-200.

Porkka-Heiskanen T (1999) Adenosine in sleep and wakefulness. Ann Med 31:125-129.

Prediger RD, Pamplona FA, Fernandes D, Takahashi RN (2005) Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) -- the spontaneously hypertensive rat (SHR). Int J Neuropsychopharmacol 8:583-594.

Ravassard P, Pachoud B, Comte JC, Mejia-Perez C, Scote-Blachon C, Gay N, Claustrat B, Touret M, Luppi PH, Salin PA (2009) Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic

transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. Sleep 32:227-240.

Rechtschaffen A, Bergmann BM (2002) Sleep deprivation in the rat: an update of the 1989 paper. Sleep 25:18-24.

Richter-Levin G, Canevari L, Bliss TV (1995) Long-term potentiation and glutamate release in the dentate gyrus: links to spatial learning. Behav Brain Res 66:37-40.

Riedel W, Hogervorst E, Leboux R, Verhey F, van Praag H, Jolles J (1995) Caffeine attenuates scopolamine-induced memory impairment in humans. Psychopharmacology (Berl) 122:158-168.

Riedel WJ, Jolles J (1996) Cognition enhancers in age-related cognitive decline. Drugs Aging 8:245-274.

Rigoulot MA, Leroy C, Koning E, Ferrandon A, Nehlig A (2003) Prolonged low-dose caffeine exposure protects against hippocampal damage but not against the occurrence of epilepsy in the lithium-pilocarpine model in the rat. Epilepsia 44:529-535.

Ritchie K, Carriere I, de Mendonca A, Portet F, Dartigues JF, Rouaud O, Barberger-Gateau P, Ancelin ML (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). Neurology 69:536-545.

Rosenzweig ES, Barnes CA (2003) Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog Neurobiol 69:143-179.

Roussinov KS, Yonkov DI (1976) Cholinergic mechanisms in the learning and memory facilitating effect of caffeine. Acta Physiol Pharmacol Bulg 2:61-68.

Ruskin DN, Liu C, Dunn KE, Bazan NG, LaHoste GJ (2004) Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. Eur J Neurosci 19:3121-3124.

Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, Kushiya E, Yagi T, Aizawa S, Inoue Y, Sugiyama H, et al. (1995) Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature 373:151-155.

Samkoff JS, Jacques CH (1991) A review of studies concerning effects of sleep deprivation and fatigue on residents' performance. Acad Med 66:687-693.

Sansone M, Battaglia M, Castellano C (1994) Effect of caffeine and nicotine on avoidance learning in mice: lack of interaction. J Pharm Pharmacol 46:765-767.

Schacter DL, Cooper LA, Valdiserri M (1992) Implicit and explicit memory for novel visual objects in older and younger adults. Psychol Aging 7:299-308.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11-21.

Shapiro ML, Eichenbaum H (1999) Hippocampus as a memory map: synaptic plasticity and memory encoding by hippocampal neurons. Hippocampus 9:365-384.

Sherry DF, Jacobs LF, Gaulin SJ (1992) Spatial memory and adaptive specialization of the hippocampus. Trends Neurosci 15:298-303.

Sherwood NT, Lo DC (1999) Long-term enhancement of central synaptic transmission by chronic brain-derived neurotrophic factor treatment. J Neurosci 19:7025-7036.

Shieh PB, Hu SC, Bobb K, Timmusk T, Ghosh A (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron 20:727-740.

Shukitt-Hale B, McEwen JJ, Szprengiel A, Joseph JA (2004) Effect of age on the radial arm water maze-a test of spatial learning and memory. Neurobiol Aging 25:223-229.

Silva AJ, Stevens CF, Tonegawa S, Wang Y (1992a) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257:201-206.

Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992b) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257:206-211.

Smellie FW, Davis CW, Daly JW, Wells JN (1979) Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. Life Sci 24:2475-2482.

Smith AP (2009) Caffeine, cognitive failures and health in a non-working community sample. Hum Psychopharmacol 24:29-34.

Smith C (1995) Sleep states and memory processes. Behav Brain Res 69:137-145.

Smith CT, Conway JM, Rose GM (1998) Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiol Learn Mem 69:211-217.

Song D, Xie X, Wang Z, Berger TW (2001) Differential effect of TEA on long-term synaptic modification in hippocampal CA1 and dentate gyrus in vitro. Neurobiol Learn Mem 76:375-387.

Springer JE, Gwag BJ, Sessler FM (1994) Neurotrophic factor mRNA expression in dentate gyrus is increased following in vivo stimulation of the angular bundle. Brain Res Mol Brain Res 23:135-143.

Squire LR (1993) The hippocampus and spatial memory. Trends Neurosci 16:56-57.

Srivareerat M, Tran TT, Alzoubi KH, Alkadhi KA (2008) Chronic Psychosocial Stress Exacerbates Impairment of Cognition and Long-Term Potentiation in beta-Amyloid Rat Model of Alzheimer's Disease. Biol Psychiatry.

Stefurak SJ, Stefurak ML, Mendelson WB, Gillin JC, Wyatt RJ (1977) A method for sleep depriving rats. Pharmacol Biochem Behav 6:137-139.

Stickgold R (2005) Sleep-dependent memory consolidation. Nature 437:1272-1278.

Stones MJ (1977) Memory performance after arousal from different sleep stages. Br J Psychol 68:177-181.

Suarez LD, Smal L, Delorenzi (2010) A Updating contextual information during consolidation as result of a new memory trace. Neurobiol Learn Mem.

Suchecki D, Tufik S (2000) Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat. Physiol Behav 68:309-316.

Suchecki D, Lobo LL, Hipolide DC, Tufik S (1998) Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. J Sleep Res 7:276-281.

Sutherland GR, Peeling J, Lesiuk HJ, Brownstone RM, Rydzy M, Saunders JK, Geiger JD (1991) The effects of caffeine on ischemic neuronal injury as determined by magnetic resonance imaging and histopathology. Neuroscience 42:171-182.

Sweatt JD (1999) Toward a molecular explanation for long-term potentiation. Learn Mem 6:399-416.

Takahashi RN, Pamplona FA, Prediger RD (2008) Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. Front Biosci 13:2614-2632.

Tamamaki N, Abe K, Nojyo Y (1988) Three-dimensional analysis of the whole axonal arbors originating from single CA2 pyramidal neurons in the rat hippocampus with the aid of a computer graphic technique. Brain Res 452:255-272.

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 20:709-726.

Tartar JL, Ward CP, McKenna JT, Thakkar M, Arrigoni E, McCarley RW, Brown RE, Strecker RE (2006) Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. Eur J Neurosci 23:2739-2748.

Terry WS, Phifer B (1986) Caffeine and memory performance on the AVLT. J Clin Psychol 42:860-863.

Teyler TJ, DiScenna P (1985) The role of hippocampus in memory: a hypothesis. Neurosci Biobehav Rev 9:377-389.

Teyler TJ, DiScenna P (1986) The hippocampal memory indexing theory. Behav Neurosci 100:147-154.

Thiels E, Xie X, Yeckel MF, Barrionuevo G, Berger TW (1996) NMDA receptor-dependent LTD in different subfields of hippocampus in vivo and in vitro. Hippocampus 6:43-51.

Thomas KL, Laroche S, Errington ML, Bliss TV, Hunt SP (1994) Spatial and temporal changes in signal transduction pathways during LTP. Neuron 13:737-745.

Tiba PA, Oliveira MG, Rossi VC, Tufik S, Suchecki D (2008) Glucocorticoids are not responsible for paradoxical sleep deprivation-induced memory impairments. Sleep 31:505-515.

Tobler I, Scherschlicht R (1990) Sleep and EEG slow-wave activity in the domestic cat: effect of sleep deprivation. Behav Brain Res 37:109-118.

Tobler I, Franken P, Scherschlicht R (1990) Sleep and EEG spectra in the rabbit under baseline conditions and following sleep deprivation. Physiol Behav 48:121-129.

Tokuda M, Ahmed BY, Lu YF, Matsui H, Miyamoto O, Yamaguchi F, Konishi R, Hatase O (1997) Involvement of calmodulin-dependent protein kinases-I and -IV in long-term potentiation. Brain Res 755:162-166.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49-62.

Tran TT, Srivareerat M, Alkadhi KA (2010) Chronic psychosocial stress triggers cognitive impairment in a novel at-risk model of Alzheimer's disease. Neurobiol Dis 37:756-763.

Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 87:1327-1338.

Tully T (1997) Regulation of gene expression and its role in long-term memory and synaptic plasticity. Proc Natl Acad Sci U S A 94:4239-4241.

Van Hulzen ZJ, Coenen AM (1980) The pendulum technique for paradoxical sleep deprivation in rats. Physiol Behav 25:807-811.

van Hulzen ZJ, Coenen AM (1981) Paradoxical sleep deprivation and locomotor activity in rats. Physiol Behav 27:741-744.

Vogel GW (1975) A review of REM sleep deprivation. Arch Gen Psychiatry 32:749-761.

Voglis G, Tavernarakis N (2006) The role of synaptic ion channels in synaptic plasticity. EMBO Rep 7:1104-1110.

Vyazovskiy VV, Borbely AA, Tobler I (2002a) Interhemispheric sleep EEG asymmetry in the rat is enhanced by sleep deprivation. J Neurophysiol 88:2280-2286.

Vyazovskiy VV, Deboer T, Rudy B, Lau D, Borbely AA, Tobler I (2002b) Sleep EEG in mice that are deficient in the potassium channel subunit K.v.3.2. Brain Res 947:204-211.

Walker MP, Stickgold R (2006) Sleep, memory, and plasticity. Annu Rev Psychol 57:139-166.

Wang GP, Huang LQ, Wu HJ, Zhang L, You ZD, Zhao ZX (2009) Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. Neuroreport 20:1172-1176.

Wang JH, Kelly PT (1996) The balance between postsynaptic Ca(2+)-dependent protein kinase and phosphatase activities controlling synaptic strength. Learn Mem 3:170-181.

Wang JH, Kelly PT (1997) Postsynaptic calcineurin activity downregulates synaptic transmission by weakening intracellular Ca2+ signaling mechanisms in hippocampal CA1 neurons. J Neurosci 17:4600-4611.

Warburton DM (1995) Effects of caffeine on cognition and mood without caffeine abstinence. Psychopharmacology (Berl) 119:66-70.

Warburton DM, Bersellini E, Sweeney E (2001) An evaluation of a caffeinated taurine drink on mood, memory and information processing in healthy volunteers without caffeine abstinence. Psychopharmacology (Berl) 158:322-328.

Webb WB, Agnew HW, Jr. (1962) Sleep deprivation, age, and exhaustion time in the rat. Science 136:1122.

Winder DG, Mansuy IM, Osman M, Moallem TM, Kandel ER (1998) Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. Cell 92:25-37.

Winters BD, Saksida LM, Bussey TJ Implications of animal object memory research for human amnesia. Neuropsychologia.

Witter MP, Van Hoesen GW, Amaral DG (1989) Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. J Neurosci 9:216-228.

Xavier GF, Oliveira-Filho FJ, Santos AM (1999) Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: difficulties in "place strategy" because of a lack of flexibility in the use of environmental cues? Hippocampus 9:668-681.

Yang RH, Hu SJ, Wang Y, Zhang WB, Luo WJ, Chen JY (2008) Paradoxical sleep deprivation impairs spatial learning and affects membrane excitability and mitochondrial protein in the hippocampus. Brain Res 1230:224-232.

Yao H, Huang YH, Liu ZW, Wan Q, Ding AS, Zhao B, Fan M, Wang FZ (1998) [The different responses to anoxia in cultured CA1 and DG neurons from newborn rats]. Sheng Li Xue Bao 50:61-66.

Yin JC, Del Vecchio M, Zhou H, Tully T (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in Drosophila. Cell 81:107-115.

Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, Tully T (1994) Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell 79:49-58.

Yoo SS, Hu PT, Gujar N, Jolesz FA, Walker MP (2007) A deficit in the ability to form new human memories without sleep. Nat Neurosci 10:385-392.

Yoshimura H (2005) The potential of caffeine for functional modification from cortical synapses to neuron networks in the brain. Curr Neuropharmacol 3:309-316.

Youngblood BD, Zhou J, Smagin GN, Ryan DH, Harris RB (1997) Sleep deprivation by the "flower pot" technique and spatial reference memory. Physiol Behav 61:249-256.

Youngblood BD, Smagin GN, Elkins PD, Ryan DH, Harris RB (1999) The effects of paradoxical sleep deprivation and valine on spatial learning and brain 5-HT metabolism. Physiol Behav 67:643-649.

Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 6:2950-2967.