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THE EFFECTS OF ACUTE POSTTRAINING INJECTIONS OF
COCAINE ON SPATIAL MEMORY IN C57BL/6 MICE

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology:
General Experimental

by
Sergio Diaz Iñiguez
September 2007

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Approved by:


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ABSTRACT

Cocaine, a natural central nervous system stimulant, has been shown to facilitate memory performance on shock avoidance tasks in rodents. Cocaine's memory enhancing qualities have been attributed to its ability to increase dopamine levels in areas of the brain associated with learning and memory (i.e., hippocampus). This increase in dopamine initiates the process of protein production and the formation of new synapses via protein kinase A (PKA). Whether cocaine's memory enhancing effects are generalized to spatial memory tasks in rodents has not yet been determined. Thus, the purpose of this study was to investigate the effects of cocaine on spatial memory consolidation using the Morris water maze. Specifically, male and female C57BL/6 mice were trained on a spatial water task, and then administered a single posttraining injection of saline or cocaine (1.25, 2.5, 5.0, or 20.0 mg/kg). Spatial memory performance was evaluated 24 and 48 hr post drug injection. Immediately after the completion of behavioral testing, hippocampal tissue was extracted and assayed for PKA activity. It was hypothesized that low doses of cocaine would enhance water maze performance in both male and female mice. Also, it was hypothesized that

cocaine would increase PKA activity, when compared to saline controls. The results from the present study showed that both male and female C57BL/6 mice exhibited a similar behavioral response to cocaine. In contrast, the neurochemical response to cocaine was sex dependent with females showing increased PKA activity after cocaine administration, while males were unaffected by the cocaine treatment. Moreover, only 2.5 mg/kg cocaine was able to enhance performance on the water maze task, while PKA activity was increased by both 2.5 and 20.0 mg/kg cocaine. Taken together these data suggest that cocaine is able to enhance spatial memory consolidation for at least a 24 hr period in C57BL/6 mice and that this increase in memory performance is probably not related to increased PKA activity.

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CHAPTER ONE

INTRODUCTION

The discovery of the biological mechanisms of learning and memory appear to be more probable today given current technological advancements. To this end, researchers interested in memory dysfunction have been working on developing drugs that reverse memory impairment and that enhance cognitive abilities. Interestingly, even with a better understanding of brain functioning, agents that have been available for centuries may provide the answer to our need for memory enhancing drugs. For example, it has been reported that stimulant drugs (like methamphetamine and cocaine) display cognitive enhancing effects (Brown, Bardo, Mace, Phillips, & Kraemer, 2000; Introini-Collison & McGaugh, 1989). The memory enhancing effects of stimulant drugs have been attributed to their ability to increase dopamine levels in the brain. Neurobiological studies have demonstrated that stimulation of postsynaptic dopamine receptors leads to a biochemical cascade of events [mediated by protein kinase A (PKA)], which leads to protein production and the development of new synapses, thus enhancing memory performance (Hinoi, Balcar, Kuramoto,

Nakamichi, & Yoneda, 2002). Because cocaine increases the level of dopamine at the synapse, the present investigation will assess if posttraining injections of cocaine enhances memory consolidation of newly acquired spatial memories using the Morris water maze (MWM; Morris, 1981).

C57BL/6 Mice and Research

The recent introduction of genetic techniques in mice that model various neurodegenerative diseases, such as Alzheimer's disease, has pushed the mouse to the forefront of biomedical research. In particular, the C57BL/6 (C57) mouse has become critically important, as this strain is commonly used for genetic manipulation. C57 mouse genes can be manipulated by increasing or decreasing the amount of those specific proteins associated with neurodegenerative disorders. For example, amyloid plaques (protein accumulations associated with Alzheimer's disease in humans), which are not usually found in mice, can be introduced into their brains, thus making them useful models for experimentation. Previous behavioral work using rats (Rodriguez, Rodriguez, Phillips, & Martinez, 1993) suggested that cocaine may improve memory consolidation (an

aspect of memory): a topic not yet examined in C57 mice. Although the effects of cocaine on memory has not been definitively determined, it is important to further study this issue in C57 mice to better understand the underlying mechanisms of spatial memory. In addition to assessing the behavioral effects of cocaine on spatial memory, PKA activity will be evaluated in the hippocampus (a brain structure important for spatial memory performance) in order to better understand its role in spatial memory consolidation in male and female C57 mice.

CHAPTER TWO

MEMORY

Review

Memory has always been a very difficult topic to study. Part of the problem is that memory can be subdivided into many types, each with different processing mechanisms and neural substrates. The following paragraphs will review how memory is currently conceptualized and what is known about the neuroanatomical basis of declarative memory.

The first clear subdivision of memory is between declarative and non-declarative memory (see Figure 1). This memory division was first recognized because of various human clinical cases, where victims of serious accidents lost their memory [see the case of H. M. cited in Milner (1959)], and cases of individuals with abnormally elevated abilities related to learning and memory (see the case of "S" in Luria, 1968).

Declarative memory includes facts (i.e., "George Washington was the first president of the United States") and events (i.e., what you ate for lunch today).

Declarative memory is also known as "explicit" memory, given that it requires a "conscious" recollection of the remembered event (Bitan & Karni, 2004). We also remember other things in addition to facts and events. For example, a person may not have a clear and distinct memory of the day he or she learned to drive a car, yet this person is able to drive to work and to other places on a daily basis. This type of memory falls into the category of non-declarative memory. More specifically, this type of memory is referred to as "procedural memory" (memory for skills, behaviors, and habits; Bitan & Karni, 2004). Non-declarative memory can be further divided into procedural, associative (classical conditioning and operant conditioning), and non-associative (sensitization and habituation) memory (see Figure 1). Non-declarative memory is also called "implicit" memory, because it does not require a "conscious" recollection of the event (i.e., not remembering the day you learned to drive a car, yet you can drive a car to work; Gupta & Cohen, 2002). Although there is no clear limit to the number of declarative and non-declarative memories that can be stored, human studies suggest that the storage capacity of declarative memories is very high (see Luria, 1968).

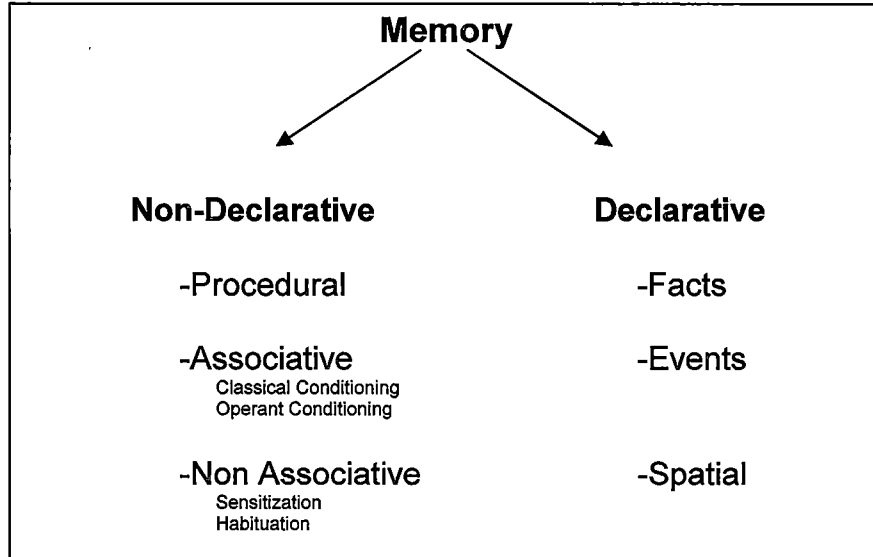


Figure 1. Subdivisions of Non-Declarative and Declarative Memory

Short-Term and Long-Term Memory

Memory can be further distinguished in terms of its duration. Short-term memory is defined as those memories that can only be recalled within seconds, minutes, or hours, and which can be easily disrupted (or lost) if they are not rehearsed at all times. A common example of short-term memory is when a person is asked to remember a phone number. If this person does not keep rehearsing the numbers in his/her mind, the information will be lost and the individual will forget the number. Interestingly,

short-term memory has a limited capacity (Glassman, Leniek, & Haegerich, 1998; Shiffrin & Nosofsky, 1994), which is seven-plus-or-minus-two bits of information. If the information is retained long enough in short-term memory, the memory will be consolidated and transferred to long-term memory. On the other hand, long-term memories can be retrieved days, months, or years after they were originally formed, and there is no known limit to the number of memories that can be stored. For example, a person can easily recall what he/she had for dinner last night (recently formed memory), and also have vivid memories of his/her childhood (memory formed decades ago).

Both short- and long-term memory can be used simultaneously, in what is termed working memory. Working memory is a limited capacity system where information is both manipulated and stored while accomplishing a specific task (Smith & Jonides, 1999). Once the task is completed, the memory must be forgotten or it will interfere with future performance (so it is not long lasting). Working memory is dependent of rules in which specific stimuli (reference points) must remain constant while other stimuli or responses are frequently changing. For example, when a student drives to school, he/she will usually park in a

different parking space in one of two different parking lots (Parking lot A or B). In parking lot A there is an abundance of trees, whereas there are none in parking lot B (reference points from long-term memory). Another reference point for the student is the color, make, and model, of his/her vehicle (i.e., red toyota camry). Since the student usually does not park his car in the same parking space nor parking lot (changing factors), he/she faces the problem of having to remember where he/she parks every day after class (problem to solve). Working memory is the process in which the student must recall whether he/she parked in lot A or B (were there trees present or not at the parking lot?), and then he/she must look for his red toyota camry within that parking lot (since the parking space also changes on a daily basis). All of this information must be retained for a specific number of hours (while the student is in class), so that he/she will successfully locate the vehicle after walking out of class. The following day, the student once again parks his/her car in a different space, so the information from the previous day is discarded, or the student will look for the car in the wrong parking lot.

Memory and the Brain

The medial temporal lobe is important for the consolidation of declarative memories (see the case of H.M. in Milner, 1959). Specifically, researchers have found that the hippocampus (a structure within the temporal lobe) is involved in a wide range of memory tasks, including working and spatial memory (Marighetto, Micheau, & Jaffard, 1993). Several studies using rodents confirm that the hippocampus is critical for storing long-term memories; however, once these memories have been consolidated, they depend on the cerebral cortex rather than the hippocampus (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999). Furthermore, the hippocampus appears to be more important for storing declarative than non-declarative memories (Cohen, Eichenbaum, Deacedo, & Corkin, 1985).

Squire (1992) suggested that the hippocampus is critical for declarative (explicit) memory in both animals (monkeys and rats) and humans. To demonstrate that damage to the hippocampus in animals is similar to humans, Zola, Squire, Teng, Stefanacci, Buffalo, and Clark (2000) used a delayed nonmatching-to-sample task with monkeys. In this task, the monkey sees an object (the sample) and after a

delay it must choose the object that does not match the sample. Zola and colleagues damaged limited areas of the hippocampus and found that the monkeys were impaired on the delayed-nonmatching-to-sample task, providing supporting evidence of the importance of the hippocampus for explicit memories (Zola et al., 2000). Moreover, numerous other animal studies support the hypothesis that the hippocampus is a critical structure for declarative memory (for review see Kesner & Hopkins, 2006).

Spatial Memory and the Hippocampus

Research involving the hippocampus increased dramatically with the discovery that this structure was important for declarative memory in humans (Milner, 1959). Researchers quickly found that animals not only display declarative memory deficits (when damage is induced to this structure) just as humans do, but also that this structure is critical for spatial memory (Bliss & Collingridge, 1993). Briefly, spatial memory is the ability to remember explicitly (declarative memory) the topographical location of one object with relation to the location of other objects (either from short, long, and/or working memory) in a given environment. For example, in the case of H.M.

(Milner, 1959), one of the devastating memory deficits after bilateral removal of his temporal lobes (including hippocampus) was the loss of spatial memory. H.M was not able to learn neither his way around the hospital he was being treated at nor his way around his new house after leaving the hospital.

Animal studies have helped clarify the role that the hippocampus plays in spatial memory. A very important discovery was made by O'Keefe and Dostrovsky (1971), who recorded the activity of pyramidal cells within the hippocampus as a rat moved around its environment. O'Keefe and Dostrovsky found that some of these neurons fired at different rates while the animal was in different locations of a maze (which they called "place cells"). In other words, the researchers found that some neurons fired at a high rate only when the rat was in a particular location (see also O'Keefe & Burgess, 1996). For example, when a rat is placed in a radial arm maze, the place cells in the hippocampus respond differently to objects outside the maze (such as a window) in relation to the arm in which they are located (these cues are usually referred to as distal or extramaze cues). Any changes to these extramaze cues, such

as placing a black curtain around the maze (thus eliminating the extramaze cues) will disrupt the rat's performance (see D'Hooge & De Deyn, 2001). Similar results have been obtained using different spatial memory paradigms, such as on the MWM (Morris, Garrud, Rawlings, & O'Keefe, 1982).

Conclusion

The study of memory has been an extensive and complicated journey for researchers, especially given its complexity and limited number of clinical human cases that exist to unveil the architecture and components of memory. Memory has been categorized into two major subdivisions referred to as declarative (conscious recollection of facts and events) and non-declarative memory (unconscious recollection of skills, behaviors, and habits). With the development of new research methods and technology, the study of memory has started to reveal how different structures in the brain affect different types of memory. For example, both human and animal studies suggest that declarative memory (conscious recollection of facts and events) and spatial memory (perception of spatial location) are highly dependent on the hippocampus.

CHAPTER THREE

DOPAMINE

Overview

Communication between neurons is mediated via endogenous chemicals (i.e., neurotransmitters and hormones). Among the chemicals known to be neurotransmitters is a small group of monoamines, which include dopamine.

From the moment dopamine was discovered over 50 years ago, it has been the subject of much research and has been found to be involved in both motor and rewarded behavior (for review, see Nieoullon, 2002). The earliest publications primarily focused on the positive correlation between the amount of striatal dopamine depletion and motor deficits observed in Parkinson's disease (Bernheimer, Birkmayer, Hornykiewicz, Jellinger, & Seitelberger, 1973). This discovery led to the development of L-DOPA therapy (among other medications to improve the symptoms of Parkinson's disease). Also, the use of dopamine antagonists in the mentally ill led to the suggestion that alterations in dopaminergic transmission may be linked to

schizophrenia (Swerdlow & Koob, 1987). Furthermore, a relationship between dopamine and reinforcement processes (particularly with abused drugs) have been discovered (Volkow, Wang, Telang, Fowler, Logan, Childress, Jayne, Ma, & Wong, 2006). For example, studies have suggested that dopamine is a key neurotransmitter in cocaine dependency (Volkow, et al., 2006). Even more interesting, dopamine has been suggested to affect memory performance in different research paradigms, where its depletion and augmentation may suggest a positive correlation with memory performance (Beatty & Rush, 1983; Luine, Bowling, & Hearn, 1990).

Biosynthesis and Catabolism of Dopamine

The synthesis of dopamine starts with phenylalanine (an essential amino acid obtained from our diet) which is metabolized into tyrosine. Tyrosine is then converted to L-DOPA by the enzyme tyrosine hydroxylase (by adding a hydroxyl group: OH- an oxygen atom and a hydrogen atom) (Hyland, 1993). Finally, L-DOPA in turn is converted to dopamine by the enzyme DOPA decarboxylase (by removing a carboxyl group: COOH- one carbon atom, two oxygen atoms, and one hydrogen atom) (Hyland, 1993). Once dopamine has

been synthesized within the terminal button, it is then transported into vesicles by vesicular transporters (Sulzer, Sonders, Poulsen, & Galli, 2005). When stimulated, the vesicles then merge into the active zone of the presynaptic terminal, releasing the neurotransmitter into the synaptic cleft (Hyland, 1993).

Once in the synapse, dopamine binds to presynaptic and postsynaptic receptors and then is pumped back into the presynaptic terminal by a presynaptic protein called a dopamine transporter (Giros & Caron, 1993). Active reuptake by the dopamine transporter reduces receptor stimulation (by decreasing the amount of dopamine at the synapse) and also decreases the amount of new dopamine synthesis required to replenish vesicular dopamine stores (Giros & Caron, 1993).

Dopamine is metabolized by two enzymes: monoamine oxidase (MAO), which is located intracellularly (i.e., on the outer membrane of mitochondria) and catechol-o-methyltransferase (COMT), which is located extracellularly (see Peyrin & Dalmaz, 1975; Trendelenburg, 1990). MAO metabolizes dopamine within the terminal button, while COMT

inactivates the excess dopamine in the extracellular environment not removed by the dopamine transporter.

The excess dopamine within the terminal button is first deaminated by MAO turning it into 3,4-dihydroxyphenylacetaldehyde (DHPA). DHPA is then oxidized by aldehyde dehydrogenase and turned into 3,4-dihydroxyphenylacetic acid (DOPAC), which after leaving the terminal is methylated to form homovanillic acid (HVA) (see left pathway on Figure 2). In the extracellular environment, dopamine is converted to 3-O-methyldopamine by COMT. MAO then turns 3-O-methyldopamine into 3-methoxy-4-hydroxyphenylacetaldehyde (MHPA) (see right pathway on Figure 2). Lastly, aldehyde dehydrogenase turns MHPA into the metabolite HVA (for review, see Tsunoda, 2006).

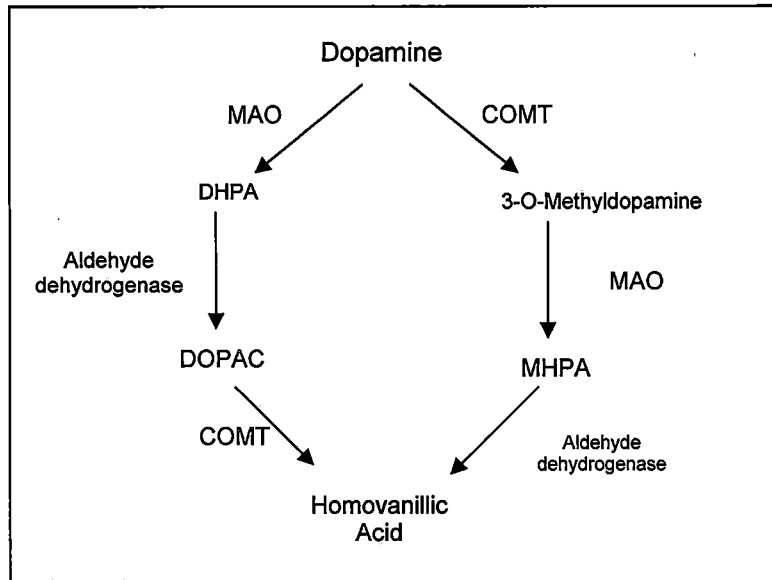


Figure 2. Catabolism of Dopamine

Dopamine Receptors

When dopamine is released from the presynaptic neuron into the synapse, it binds to specific proteins called receptors. Several receptor subtypes have been identified on dopaminergic neurons and postsynaptic terminals of gamma-aminobutyric acid (GABA) and acetylcholine (ACh) neurons. Specifically, two major dopamine receptor subtypes have been identified: D₁-like (postsynaptic) and D₂-like receptors (both presynaptic and postsynaptic on dopamine neurons) (for review, see, Nieoullon & Amalric,

2002). Under the D₁ classification, there are two subtypes: D₁ and D₅. Under the D₂ classification, there are three subtypes: D₂, D₃, and D₄ (Nieoullon & Amalric, 2002).

All dopamine receptors (D₁- and D₂-like) are metabotropic (G-protein coupled second messenger systems), which have been suggested to be responsible for long term changes in the nervous system and/or mediate long lasting changes in neural functioning, including memories (Munton, Vizi, & Mansuy, 2004; Nieoullon & Amalric, 2002).

Depending on what G-protein coupled receptor is stimulated (D₁- or D₂-like receptor) dopamine will either have a stimulatory (mediated by a G_s protein) or inhibitory (mediated by a G_i protein) effect on a biochemical cascade (see Chapter 4) that is responsible of producing proteins and long term changes in the nervous system (Munton et al., 2004).

Dopamine Pathways

Dopamine is primarily produced by two small nuclei located in the tegmentum of the midbrain: the substantia nigra and the ventral tegmental area. These nuclei project to several different forebrain areas which make up three major dopamine pathways (nigrostriatal, mesolimbic, and

mesocortical) responsible for most of the dopamine produced in the brain (Swanson, 1982).

The nigrostriatal dopamine pathway originates from cell bodies in the substantia nigra and projects to the neostriatum: the caudate nucleus and the putamen (see Figure 3). The neostriatum is important for the control of movement (Bezard, Dovero, Prunier, Ravenscroft, Chalon, Guilloteau, Crossman, Bioulac, Brotchie, & Gross, 2001), and research suggests that degeneration of these dopaminergic neurons causes Parkinson's disease (a movement disorder that causes rigidity of the limbs; Bezard et al., 2001).

Cell bodies of neurons of the mesolimbic system are located in the ventral tegmental area and project to several parts of the limbic system (nucleus accumbens, amygdala, and hippocampus; see Figure 3). Specifically, the nucleus accumbens is important for the rewarding effects of drugs such as cocaine and amphetamine (Di Ciano, Coury, Depoortere, Egilmez, Lane, Emmett-Oglesby, Lepiane, Phillips, & Blaha, 1995), and the hippocampus, as was discussed previously (see Chapter 2), is important for declarative memory consolidation (Milner, 1959).

The mesocortical pathway also originates from cell bodies in the ventral tegmental area. The axons then project to the frontal cortex. This pathway has been found to facilitate the formation of short-term memories, planning, and problem solving (Bontempi et al., 1999).

Because all of these pathways have been associated with learning, memory, reinforcement, and reward, researchers have postulated that chemical and/or structural changes to these pathways could, in turn, affect memory.

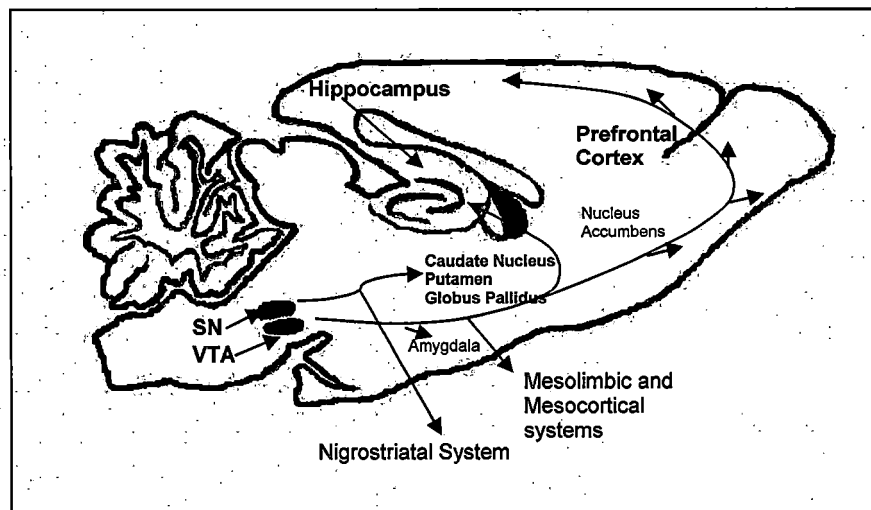


Figure 3. Schematic of Dopamine Pathways

Conclusion

In summary, dopamine is a neurotransmitter that is implicated in movement, attention, learning, and drug addiction (Wise, 1996). Three different pathways produce most of the dopamine in the brain (nigrostriatal, mesocortical, and mesolimbic). Once at the synapse, dopamine interacts with D₁- and D₂-like receptors. Then, it is mostly pumped back into the presynaptic terminal by the dopamine transporter (active reuptake process) or deactivated by COMT at the synaptic cleft. Once inside the presynaptic terminal, the enzyme MAO breaks it down into an inactive chemical (HVA).

CHAPTER FOUR

DOPAMINE AND THE PHYSIOLOGY OF MEMORY

Overview

In this chapter, the cellular mechanisms that contribute to learning and memory will be discussed. Specifically how synaptic plasticity within circuits of the hippocampus may contribute to the storage of spatial memory, and how dopamine may play an important role in this process.

Synaptic Plasticity and the cAMP-Pathway

In 1966, Lomo suggested that electrical stimulation of specific circuits in the hippocampus induces long-term synaptic changes that may be responsible for learning. Specifically, electrical stimulation of the CA1 region of the hippocampus (connected to CA3 region via schaffer collaterals) induces a long-lasting increase in magnitude of excitatory postsynaptic potentials called long term potentiation (LTP). This strengthening of synaptic transmission is due to an increase of glutamate release by the presynaptic neuron as well as an increase in the number of postsynaptic receptors (Bliss & Collingridge, 1993).

Two different postsynaptic receptors for glutamate are required for LTP to be induced, N-methyl-D-aspartate (NMDA) and non-NMDA (AMPA) receptors (Bliss & Collingridge, 1993). Recently, Kandel (2001) has also suggested that the physiological aspects of memory include: strengthening synaptic connections (LTP), gene transcription, synthesis of new proteins, and growth of new synapses.

LTP has been associated with learning and memory for two primary reasons. First, LTP is long lasting like memory (Frost, Castellucci, Hawkins, & Kandel, 1985), and second, because LTP, like many types of memory, will be attenuated or not occur if an NMDA receptor blocker (such as MK-801) is present (Heale & Harley, 1990).

Interestingly, stimulation of D₁-like dopamine receptors enhances LTP by increasing the number of AMPA and NMDA receptors in several parts of the brain that have been linked to reinforcement (nucleus accumbens; Gurden, Tassin, & Jay, 1999) and memory (the hippocampus and prefrontal cortex; Gurden, Takita, & Jay, 2000). The second messenger cyclic adenosine monophosphate (cAMP) is presumed to be the mechanism through which LTP is induced after D₁-like

receptor stimulation (Gurden et al., 2000; Jay, Gurden, & Yamaguchi, 1998).

Specifically, when dopamine binds to D₁-like receptors the membrane bound protein adenylyl cyclase is activated by a G_s protein (see Figure 4) and this causes the production of cAMP. cAMP activates PKA, which in turn recruits another protein kinase, the mitogen-activated protein kinase (MAPK), and both activate a transcriptional cascade in the nucleus (for a review, see Kandel, 2001). This transcriptional cascade starts with cAMP-response-element binding-protein-1 (CREB-1) along with the cAMP-response-element (CRE) that, in turn, promotes the transcription of target genes (the enzyme ubiquitin carboxy-terminal hydrolase and the transcription factor C/EBP) which are necessary for the growth of new synaptic connections (Bailey & Kandel, 1993; Hotte, Thuault, Lachaise, Dineley, Hemmings, Nairn, & Jay, 2006).

The cAMP transcriptional cascade is involved in different types of learning and memory, such as sensitization in aplysia (Schacher, Castellucci, & Kandel, 1988), classical conditioning in fruit flies (Mayford & Kandel, 1999) and spatial memory in rodents (Gurden et al.,

2000; Hotte et al., 2006). These findings in general suggest that dopamine is a modulator (via the cAMP pathway) in synaptic plasticity (LTP) and memory consolidation of both non-declarative and declarative memories (see Kandel, 2001).

Effects of Dopamine at the Cellular Level

The physiological effects of stimulating D₁- and D₂-like receptors are mediated through G-proteins (Sealfon & Olanow, 2000). This suggests that the effects of dopamine are slow and long lasting. Specifically, the binding of dopamine to D₁-like receptors in the hippocampus induces LTP (Gurden et al., 2000; Thompson, Gosnell, & Wagner, 2002; Ungless, Whistler, Malenka, & Bonci, 2001) and increases the production of the second messenger cAMP. As a consequence, D₁-like receptor stimulation induces a transcriptional cascade in the cell nucleus (see Figure 4) that leads to the production of new proteins and synapses (as described above).

On the other hand, the binding of dopamine to D₂-like receptors (on postsynaptic terminals) activates an inhibitory G-protein (G_i) that decreases cAMP by suppressing the activity of adenylyl cyclase (Adell & Artigas, 2004).

Also, the binding of dopamine to D₂-like receptors on the presynaptic terminal of dopamine neurons (autoreceptors) decreases cAMP via the second messenger diacylglyceral (Adell & Artigas, 2004).

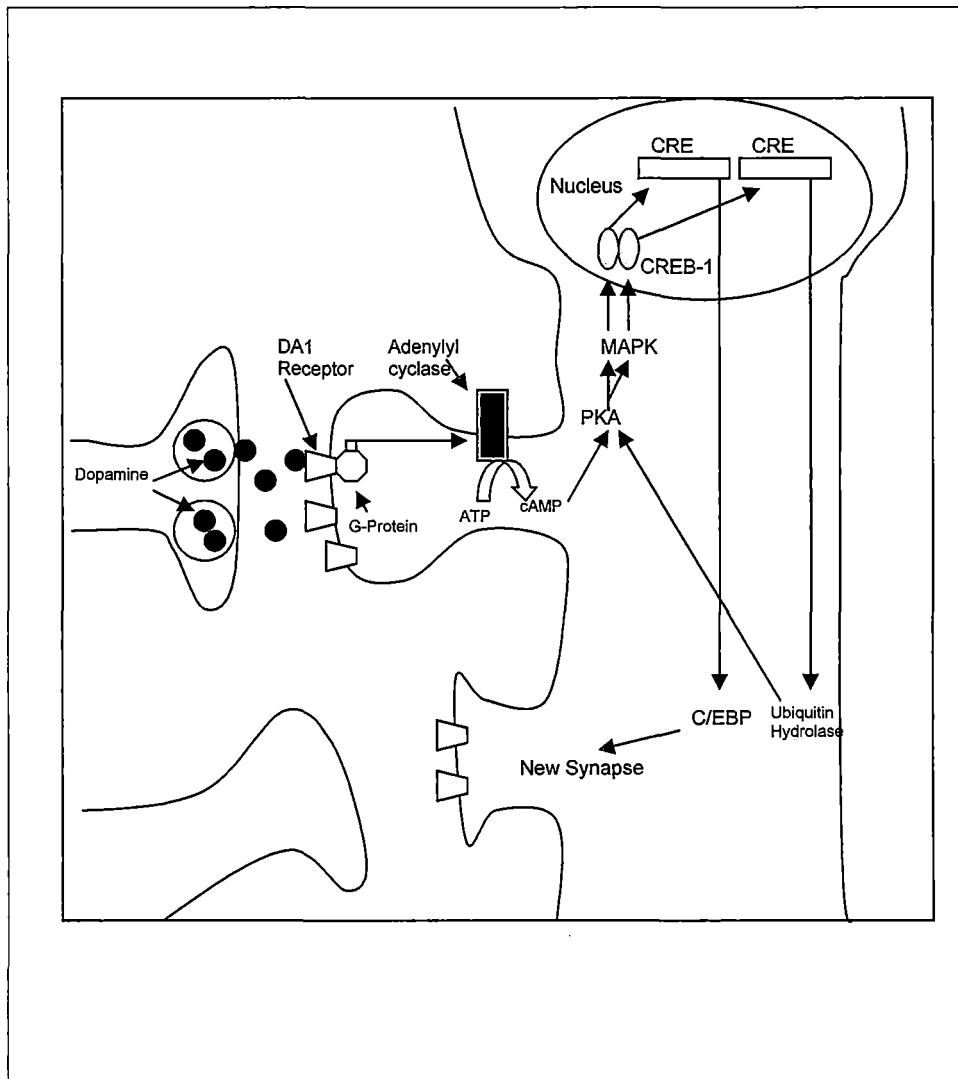


Figure 4. Dopamine Activated cAMP-PKA Biochemical Cascade

Conclusion

The physiological effects of dopamine on the postsynaptic terminal are directly linked to the second messenger protein that it binds to. Importantly, when dopamine binds to postsynaptic D₁-like receptors in the hippocampus, it starts a long-lasting transcriptional cascade that leads to the growth of new synapses via the cAMP pathway. Also, dopamine enhances LTP in the hippocampus and prefrontal cortex (Hotte et al., 2006), suggesting that dopamine can modulate the consolidation of spatial memories. For that reason, drugs that affect the dopamine systems (i.e., cocaine) may be useful tools in understanding and clarifying the role that dopamine plays as a modulator of memory.

CHAPTER FIVE

COCAINE AND MEMORY

Cocaine is a stimulant that increases the amount of dopamine in the synaptic cleft by inhibiting presynaptic dopamine transporters (for review, see Anderson & Pierce, 2005). This increase in dopamine in the nucleus accumbens and the striatum is known to be important for the addictive and locomotor stimulating properties of cocaine (Koob & Nestler, 1997). In addition to increasing dopamine in the above mentioned brain regions, cocaine also increases synaptic dopamine and other monoamine levels in the hippocampus (Pothos, 2002). Given that increased synaptic levels of monoamines are known to enhance learning and memory performance (Luine et al., 1990), cocaine administration would be expected to improve memory. This idea is supported indirectly by studies showing that chronic cocaine administration increases memory associated proteins in the hippocampus (Thompson et al., 2002). Specifically, it has been shown that administering 45 mg/kg cocaine (in a 14 day binge model) increases glutamate

receptors and PKA, which are important for LTP (Freeman, Brebner, Lynch, Robertson, Roberts, & Vrana, 2001).

Behavioral studies examining whether cocaine improves memory performance have provided mixed results. In the following paragraphs, past research assessing the effect of cocaine on memory performance will be discussed, with particular attention paid to the importance of dose, timing, and number of drug administrations.

Acute Posttraining Administration of Cocaine and Memory

Since memory storage is known to be affected by post-training manipulation of dopaminergic systems (Castellano, Cestari, Cabib, & Puglisi-Allegra, 1993), cocaine has been administered acutely after training in order to determine if it enhances memory consolidation processes. For example, Introini-Collison and McGaugh (1989) trained CFW male mice in a one-trial inhibitory avoidance task followed immediately by a posttraining injection of cocaine. Introini-Collison and McGaugh re-tested the subject's memory 24 hours after the drug administration. In order to determine if the effects of cocaine on memory are time dependent, a second group of mice received a cocaine

injection 60 minutes after the completion of training. Their findings suggested that cocaine (0.1 mg/kg) did indeed enhance memory performance. Also, the memory enhancing effects of cocaine were time dependent, because mice administered cocaine 60 minutes after completing the task did not differ from controls. The dose response curve was in the shape of an inverted-U, with the higher doses (0.3 and 1.0 mg/kg) being ineffective. Similarly, Castellano, Zocchi, Cabib, and Puglisi-Allegra (1996) found that posttraining injections of cocaine (2.5 and 5.0 mg/kg) enhanced memory performance on a one-way avoidance task using C57 male mice (see also Ciamei, Cestari, & Castellano, 2000). Interestingly, similar results have been reported in male rats, although, a 5 mg/kg dose of cocaine enhanced memory performance (Janak, Keppel, & Martinez, 1992). Similar to the Introini-Collison and McGaugh (1989) study using mice, Janak and colleagues (1992) showed that the effects of cocaine could be represented by an inverted-U shape curve, with both lower (2.5 mg/kg) and higher (7.5 mg/kg) cocaine doses being ineffective. The effects of cocaine were time dependent, because the treatment was only effective if administered immediately after training.

Chronic Cocaine Administration and Memory

In contrast to studies where cocaine was given acutely, chronic administration of cocaine generally impairs memory performance. For example, a 10-day administration of cocaine (5.6-19.0 mg/kg) prior to testing was found to impair operant-conditioning memory tasks in rats (Janak, Rodriguez, & Martinez, 1997; but see Taylor & Jentsch, 2001). Spatial memory was also impaired or delayed in rats when cocaine (20-40 mg/kg) was administered eight consecutive days prior to testing on the MWM (Quirk, Richards, & Avery, 2001). These studies suggest that chronic pretreatment with cocaine impairs (or delays) memory consolidation processes. While the reason for this impairment is not clear, high doses of cocaine (over prolonged periods of time) may lead to neurotoxicity (Levin, 1993), which in turn may lead to impaired memory performance on behavioral tasks.

Cocaine and Sex Effects

An extended body of literature describing the behavioral effects of cocaine in both humans and rodents suggests that cocaine induces behavioral differences in

males and females (Festa, Russo, Gazi, Niyomchai, Kemen, Lin, Foltz, Jenab, & Quinones-Jenab, 2004). In general, females have been reported to be more sensitive to the effects to cocaine (for review see Festa and Quinones-Jenab, 2004). For example, female rats sensitize to cocaine's behavioral effects more rapidly than male rats and display greater locomotor behavior after both acute and/or chronic cocaine administration (Chin, Sternin, Wu, Burrell, Lu, Jenab, Perrotti, & Quinones-Jenab, 2002). Also, female rats administered with lower doses of cocaine, when compared to males, acquire cocaine conditioned place preference with fewer training sessions (Russo, Jenab, Fabian, Festa, Kemen, & Quinones-Jenab 2003).

Conclusion

Since memory is positively correlated with the amount of monoamines in the synapse (Beatty & Rush, 1983; Luine et al., 1990; Packard & White, 1989) and posttraining administration of dopamine agonists have been found to enhance memory consolidation (Brown et al., 2000; Castellano et al., 1996), cocaine may be a useful tool to demonstrate if increased levels of dopamine assist spatial memory consolidation in more challenging memory paradigms. While the effects of cocaine on memory are not conclusive,

there is evidence suggesting that acute administration of cocaine (in low doses) after training may enhance memory performance (White, Christensen, Flory, Miller, & Rebec, 1995; Wood, Fay, Sage, & Anagnostaras, 2007), while chronic-administration (or high dose treatments) may impair memory acquisition (Quirk et al., 2001). Therefore, there is suggestive evidence indicating that a single posttraining injection of cocaine may assist spatial memory performance in C57 mice after MWM training. Interestingly, the limited literature on the effects of cocaine on memory does not include female subjects. This omission is unfortunate since cocaine has been found to affect male and female rodents behaviorally in different ways (Chin, Sternin, Fletcher, Jenab, Perrotti, & Quinones-Jenab, 2001). Specifically, female rodents usually are more sensitive than males to cocaine-induced psychomotor stimulation (i.e., greater locomotor-, ambulatory-, and rearing-activity; Chin et al., 2002; Festa et al., 2004) and therefore cocaine may affect their spatial memory consolidation differently as well. For this reason, one of the goals of this investigation is to also assess the effects of posttraining injections of cocaine on spatial memory consolidation between male and female C57 mice.

CHAPTER SIX

ASSESSING SPATIAL MEMORY

Over the years, several different animal research paradigms have been introduced to study spatial memory. In the following paragraphs, the two most widely used tasks to study spatial memory in rodents will be discussed.

Radial Arm Maze

In 1976, Olton and Samuelson introduced the radial arm maze for the study of spatial memory. The radial arm maze consist of eight arms radiating from a focal middle point (in which the rat is placed), and food-pellets (reward) are placed at the end of the arms (see Figure 5). Eventually, through extensive training, the rats learn to visit every arm (to retrieve the reward) without re-entering a previously visited arm. This learning pattern is an example of spatial memory, because rats remember their spatial location in reference to the previously visited arms and not as a result of odor markings, intra-maze cues, or visiting the arms in a specific pattern (Olton & Samuelson, 1976). Overall, the radial arm maze has been used widely as a method for testing spatial memory tasks

(including spatial working and reference memory) for different research purposes. For example, Olton and Papas (1979) developed a version of this maze to simultaneously test spatial working and reference memory. In this version of the radial arm maze, only four maze arms are baited (the same ones each day), and the rats learn to avoid the four arms that never have food (this is the reference memory part, thus entry to one of this arms is considered a reference memory error). Within the training sessions of each day, re-entry to one of the baited arms is then considered a working memory error.

Because learning the spatial task of the radial arm maze is dependent on the rodent's ability to collect food pellets (a form of appetitive instrumental conditioning) a major problem of using the radial arm maze is that animals may require extensive training sessions to initially learn the task (sometimes more than 20 trials). The reason for the extended training has been attributed to stress, satiation, and/or motivational factors (Miller & Dess, 1996). For example, because the radial arm maze is elevated and the arms are open (rodents do not like open spaces), stress may influence the rodents performance and may bias the results of spatial memory testing (Luine,

Villegas, Martinez, & McEwen, 1994). Similarly, the rodents may not be hungry (if food and water is available ad lib in their homecage), and therefore may not be motivated to complete the task. Therefore, the animals may have to be food-deprived in order to ensure that they are learning the task effectively, which can also negatively affect spatial learning tasks (Beck & Luine, 1999; Miller & Dess, 1996). Although the radial arm maze is a well established experimental method for studying spatial memory, researchers must be cautious and control several factors (in addition to their designed independent variables), such as satiation, food deprivation, motivation, and/or stress, that may affect the results of their study.

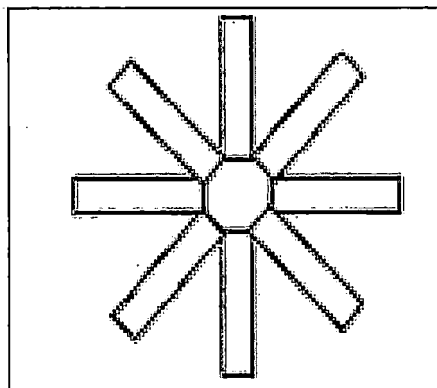


Figure 5. Schematic of an Eight Arm Radial Arm Maze

Morris Water Maze

Like the radial arm maze, the MWM is a method used to investigate spatial learning and memory in laboratory animals (Morris, 1981). It has become one of the most frequently used behavioral paradigms in neuroscience. Since its introduction, the MWM has been widely used in the validation of several rodent models for neurocognitive disorders in addition to the study of spatial learning and memory (D'Hooge & De Deyn, 2001).

The MWM is a circular water tank, in which an escape platform is submerged under water (see Figure 6). The rationale behind the MWM is that subjects will use extra-maze cues (other than the maze itself) to locate the escape platform (thus requiring the use of spatial memory, since the platform is not directly visible to the subject). This spatial memory task reduces the likelihood of the subjects using other methods than spatial memory to locate the escape platform. In specific circumstances, however, rats may use other strategies to locate the platform, such as following odor trails (Means, Alexander, & O'Neal, 1992) or by following a learned sequence of movements (Brandeis, Brandys, & Yehuda, 1989). Thus, researchers using the MWM

must plan to control of all possible variables that could affect the performance of the subjects.

The MWM can also be used to test non-spatial memory. The non-spatial version of the MWM uses a second escape platform that is directly visible to the subject by placing a flag on the platform itself. Usually, the platform is above the water and also painted with a vivid color to ensure visibility. In addition, in the non-spatial test, the MWM is surrounded with black curtains to minimize extra maze cues. This non-spatial task is usually used as a control measure, to ensure that motivational and/or sensorimotor defects do not affect or influence the spatial learning performance of the subject.

Task simplicity is one of the most common reasons why the MWM has been widely used for the study of spatial memory. This is most evident when the MWM is compared to other well established spatial memory paradigms (such as the radial arm maze), which require intensive training protocols or face motivational problems related to satiation. Satiation is not a problem with MWM, because the subject is always motivated to escape the water.

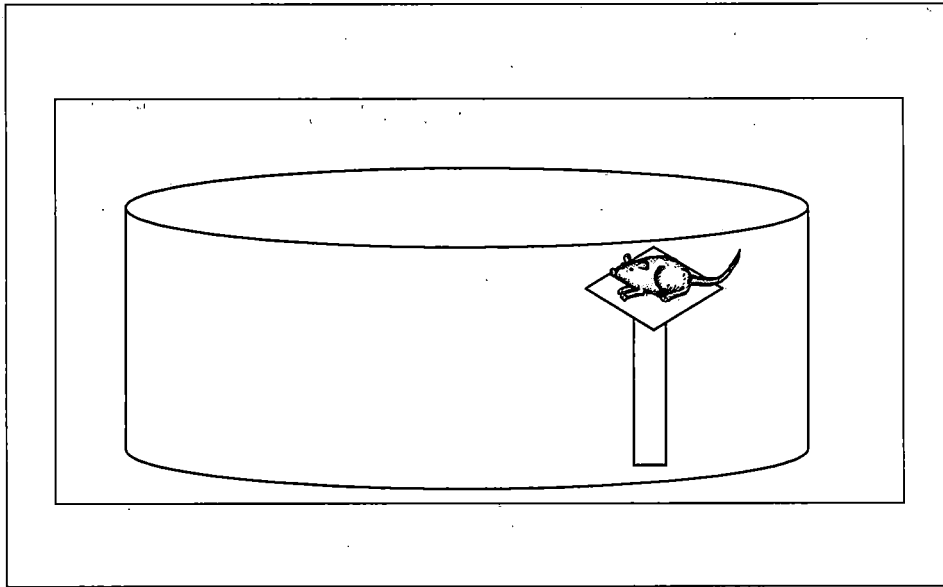


Figure 6. Schematic of a Morris Water Maze

On the other hand, one of the most noted disadvantages of the MWM is that it requires subjects to escape from an aversive stimulus (water) (Block, 1999). When the subject is initially introduced into the water tank, the subject's stress can affect its cognitive function on the maze (Holscher, 1999). To control for this problem, it has been suggested that the water should be maintained at a reasonable temperature (Stewart & Morris, 1993), and that the subject should be habituated to the water-immersion process by using a short adaptation procedure, such as a

straight-water channel; prior to the beginning of the experiment.

Conclusion

Overall, when comparing the two most widely used experimental paradigms assessing spatial memory in rodents (radial arm maze and MWM), the MWM has been the method of choice for neuroscientists, because of the quick and simple training procedures and the constant motivation provided by a natural aversion to water in many animals.

CHAPTER SEVEN

HYPOTHESIS

The purpose of the present investigation was to assess the effects of posttraining injections of cocaine on spatial memory consolidation in C57 male and female mice. To this end, the effects of cocaine on spatial memory performance were evaluated on the MWM. Memory performance was assessed by measuring the swim latency (time) and swim velocity (cm/s) to reach the escape platform.

In this experiment, C57 male and female mice underwent a three day testing period on the MWM (Gresack & Frick, 2006). The mice were given eight acquisition trials to learn the spatial memory task followed by a single acute injection of cocaine (1.25, 2.5, 5.0 or 20.0 mg/kg) or saline (conditioning day). Twenty-four and 48 hours later the mice returned to the water maze and were given a single swim trial in order to test their memory retention of the location of the hidden escape platform. It was hypothesized that cocaine would enhance spatial memory performance, and that this effect would be gender and dose dependent. Specifically, it was hypothesized that mice administered low doses of cocaine (1.25, 2.5, and 5.0

mg/kg) would perform better on the spatial memory task (drug main effect) when compared to the mice in the saline control condition. However, mice injected with a high dose of cocaine (20.0 mg/kg) would perform worse on the spatial memory task than mice in the control condition.

Because male and female rodents have been found to respond differently to the effects of cocaine, with females displaying greater number of locomotor and rearing behaviors (Festa et al., 2004), it was hypothesized that male and female mice would display a differential memory enhancement on the MWM as a function of drug administration. In other words, in female mice, the group administered the lowest dose of cocaine (1.25 mg/kg) would display better memory performance; while male mice administered 2.5 and 5.0 mg/kg cocaine would display superior memory performance (when compared to half control groups).

Lastly, because there is evidence suggesting that protein kinases mediate memory consolidation via gene expression (Nguyen, Abel, & Kandel, 1994), the current investigation examined the effects of acute injections of cocaine on PKA systems in the hippocampus. Based on cocaine's mechanism of action, it was hypothesized that PKA

activity in the hippocampus would be dependent of cocaine administration. Specifically, that cocaine would induce an increase in hippocampal PKA activity (when compared to saline controls). It was expected that the group of mice exhibiting enhanced spatial memory performance on the MWM would also display greater hippocampal PKA activity.

CHAPTER EIGHT

METHODS

Subjects

A total of 109 C57BL/6 mice were obtained from Harlem (Madison, WI). All mice appeared in good health upon arrival to the laboratory colony. A total of 53 mice were male, while 56 were female. Mice were housed (3-4 per cage) and allowed to acclimate to the colony room for 9 days prior to handling at California State University San Bernardino (CSUSB) in a room with a temperature of 22-23°C with a 12:12 hour light/dark cycle, and with food and water accessible *ad libitum*. All mice (9 week-old) completed behavioral testing although two subjects assigned to saline group (one female and one male) were excluded from all data analyses given that their latencies on swim trial 9 (\bar{x} = 3.50 and \bar{x} = 4.17 respectively) were not representative of their group mean (\bar{x} = 23.75, \pm SEM = 3.14.). If included, Test Day 1 data would be marginally significant ($P < 0.07$). All subjects were treated according to the *Guide for the Care and Use of Mammals in Neuroscience and Behavioral*

Research (National Research Council, 2003). This project was also approved by the Institutional Animal Care and Use Committee of CSUSB (IACUC).

Apparatus

The MWM was a white circular water tank 97 cm in diameter and 58 cm in height. The water maze was filled with water to a depth of 18 cm. The water temperature was maintained at 24°C using a standard heat-lamp. Around the perimeter of the water tank, four starting points (north, south, east, west) were equally positioned, therefore, dividing the water maze into four equal quadrants. The escape platform (10 X 10 cm) was submerged to a depth of 0.5 cm on the north-east quadrant. Extramaze cues were placed throughout the walls of the testing room.

Drug Treatment

Subjects were assigned to one of five groups (9-11 mice per group), and received an intraperitoneal (IP) injection of either saline (10.0 ml/kg) or cocaine hydrochloride (1.25, 2.5, 5.0, or 20.0 mg/kg) dissolved in 0.9% NaCl (Sigma, St. Louis, MO). Specifically, mice received an injection of either saline (control group) or

cocaine immediately after the completion of spatial memory training (first eight trials, here after referred to as posttraining injections).

Procedure

Habituation (Day 1 to Day 5)

Mice were handled for five days in order to habituate them to both the experimenter and testing environment. Mice were briefly handled (5 min each time) on habituation days 1 through 5 (see Table 1). On habituation day 5, mice were also habituated to the testing room for 20 min. This procedure was followed to reduce stress by handling and exposing the mice to the testing environment. Lastly, mice were habituated to the water immersion process on day 5 (see Gresack & Frick, 2006). Briefly, mice were given 4 shaping trials. On trial 1, the mouse was placed for 10 s on the escape platform. For the remaining trials, the mouse was placed at three distances progressively further from the platform and allowed to swim to the platform. If the mouse did not find the platform within 60 s, then it was led to it by the experimenter. No data were collected during shaping.

Spatial Memory Task (Day 6 to Day 8)

The mice received one training session of eight-trials on day 6 (conditioning day). Mice were placed in the water maze at one of the four starting points (north, south, east, and west) and allowed to freely swim and find the submerged escape platform (located in the north-west quadrant). Every starting point was used twice within the eight trials. If the mouse did not locate the hidden platform within the 60 s provided, the experimenter directed the mouse to the escape platform. Once on the escape platform, the mouse was allowed 10 s to view its surroundings (to view extra-maze cues). After every trial, the mouse was dried with a towel and placed in a holding cage for a 45-second intertrial interval. At the end of the eight trials, the mouse was injected with either saline or cocaine (1.25, 2.5, 5.0, or 20.0 mg/kg), and placed back into the home cage (see Table 1). After 24 hr (day 7; test day 1), the mice were returned to the water maze for a single memory retention trial (trial 9). All mice were released from the same starting point (north point). Immediately after trial 9 (day 7), half of the subjects were killed and hippocampal tissue was extracted. The other half of mice returned to the MWM for an additional

swim trial (trial 10) on day 8 (test day 2). Once again, all mice were released from the same point (north point). After completing swim trial 10, the remaining mice were killed and hippocampal tissue was extracted. On the conditioning day (day 6) and both test days (day 7 and 8; see Table 1), latency (s) and velocity (cm/s) to find the escape platform were recorded via an automated computer tracking system (NOLDUS).

Table 1. Summary of Experiment

Day 1-5	Day 6 Conditioning Day	Day 7 Test-Day 1	Day 8 Test-Day 2
-Handling -Habituation	Spatial memory training (8-trials), followed by Drug Injection.	-1 retention trial -Tissue Extraction (half subjects)	-1 retention trial -Tissue Extraction (half subjects)

Membrane Preparation

Mice were killed by rapid decapitation immediately after behavioral testing and their hippocampi were removed on dry ice and stored at -80°C . Frozen tissue was placed in homogenization buffer (50 mM Tris (pH 7.4), 100 ng/ml aprotinin, and 5 mM EDTA) and homogenized using a hand-held

Teflon homogenizer (see Crawford, Choi, Kohutec, Yoshida, & McDougall, 2004). Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) based on the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

PKA Assay

PKA assays were performed using the method previously described by Crawford et al. (2004). Duplicate hippocampi homogenates containing approximately 4 μg of protein for each subject were incubated for 5 min at 30°C in phosphorylation buffer [50 mM Tris (pH 7.4), 10 mM MgCl_2 , and 0.25 mg/ml BSA], containing 50 μg of kemptide and 100 μg [γ - ^{32}P]ATP (ICN, Costa Mesa, CA). In addition, the buffer contained either cAMP (10 μM) or protein kinase inhibitor (PKI) (6-22) amide (1 μM /reaction). Following incubation, the phosphorylation mixture was blotted on phosphocellulose filter paper. The filter paper was washed twice with 1% phosphoric acid for 5 min, followed by two 5 min washes with double-distilled water. Filters were then placed in scintillation fluid and quantified by liquid scintillation spectrometry. PKA activity was defined as the difference

between PKA activity in the presence of cAMP and that measured in the presence of PKI.

Data Analysis

The behavioral data were analyzed using one- or two-way analyses of variance (ANOVA) for repeated measures, with experimental group (sex and drug) and swim trial (repeated measure) as sources of variance for spatial memory (Gresack & Frick, 2006). Post hoc comparisons were made using Tukey tests.

Hippocampal PKA activity was also analyzed using one- or two-way ANOVAs with drug and sex as sources of variance. Post hoc comparisons for PKA activity were made using Dunnett tests. In all cases, a significance level of $P < .05$ was adopted to determine statistical significance.

CHAPTER NINE

RESULTS

Morris Water Maze

Conditioning Day

On the conditioning day (day 6; training trials 1-8), male and female mice performed similarly, as there were no statistically significant differences in latency [time to locate the escape platform (s)] or velocity (cm/s) between the groups (see Figures 7 and 8 respectively). All mice did improve over the course of the eight training trials because a significant swim trial (repeated measure) main effect involving latency ($F_{7,679} = 4.08$, $P < 0.0001$; Tukeys) indicated that mice located the platform in less time on trials 7 and 8 as compared to trials 1 and 2 (see Figure 9A).

Although unrelated to spatial memory acquisition, swim velocity was also recorded in order to control for physical differences in swim ability between the mouse groups. A significant swim trial (repeated measure) main effect involving swim velocity ($F_{7,679} = 5.29$, $P < 0.0001$; Tukeys) indicated that swim speeds (cm/s) across the training trials changed consistently across swim trials; trials 1,

3, 5, 6, 7, and 8 were slower when compared to trial 2 (see Figure 9B). Overall, the data from the conditioning day suggests that all mice learned the location of the platform in a similar fashion, because both latencies and swim speeds decreased regardless of group (across drug group assignment and sex).

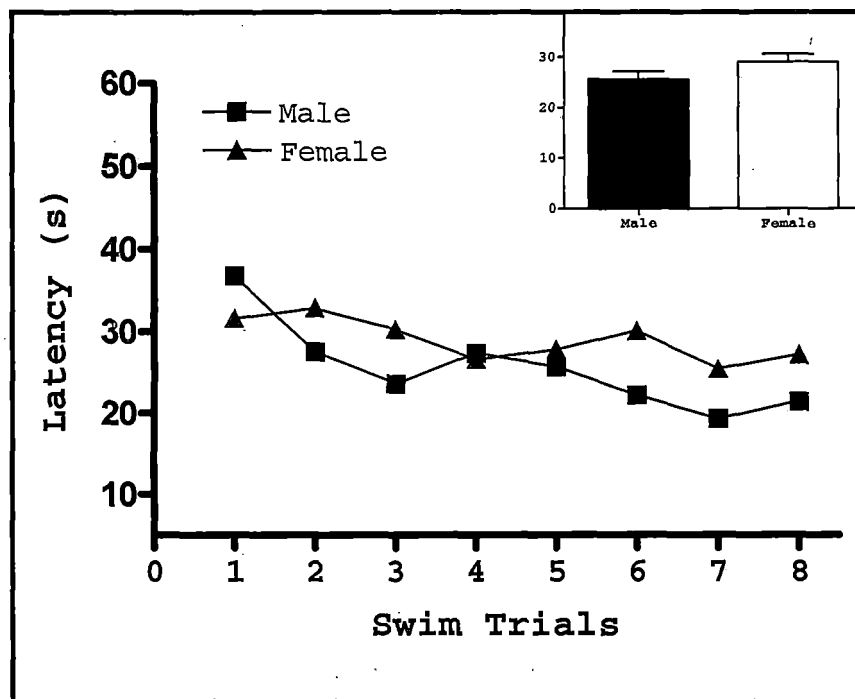


Figure 7. Mean Swim Latency (s) to Locate Escape Platform for Male and Female C57 Mice Across the Eight Training Trials on the MWM on the Conditioning Day (Day 6)

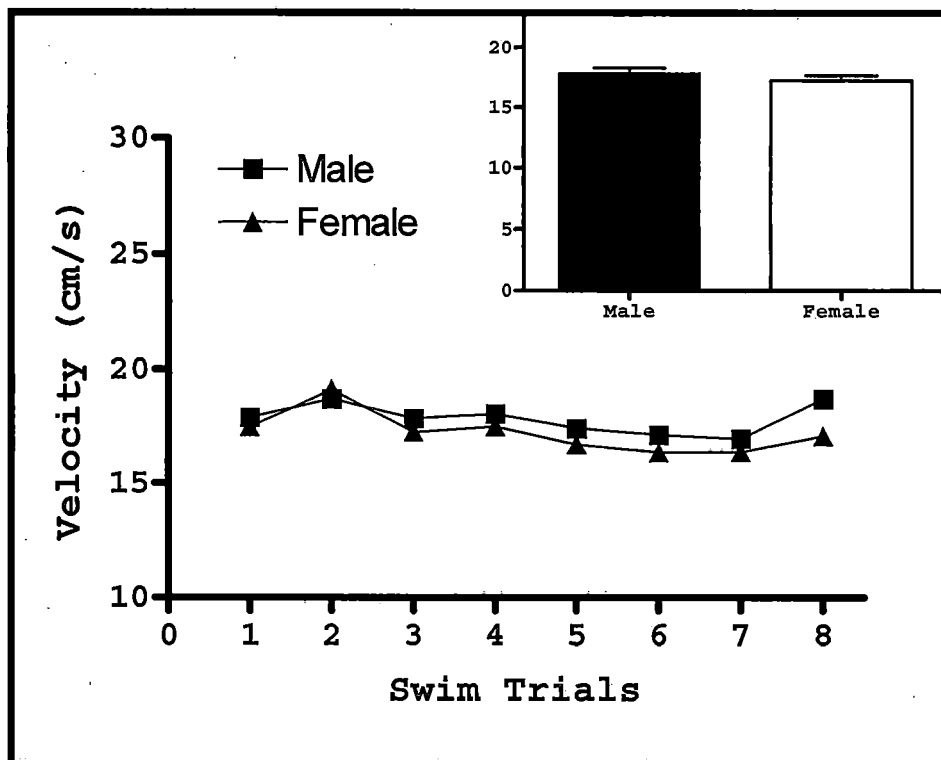


Figure 8. Mean Swim Velocity (cm/s) to Locate Escape Platform for Male and Female C57 Mice Across the Eight Training Trials on the MWM on the Conditioning Day (Day 6)

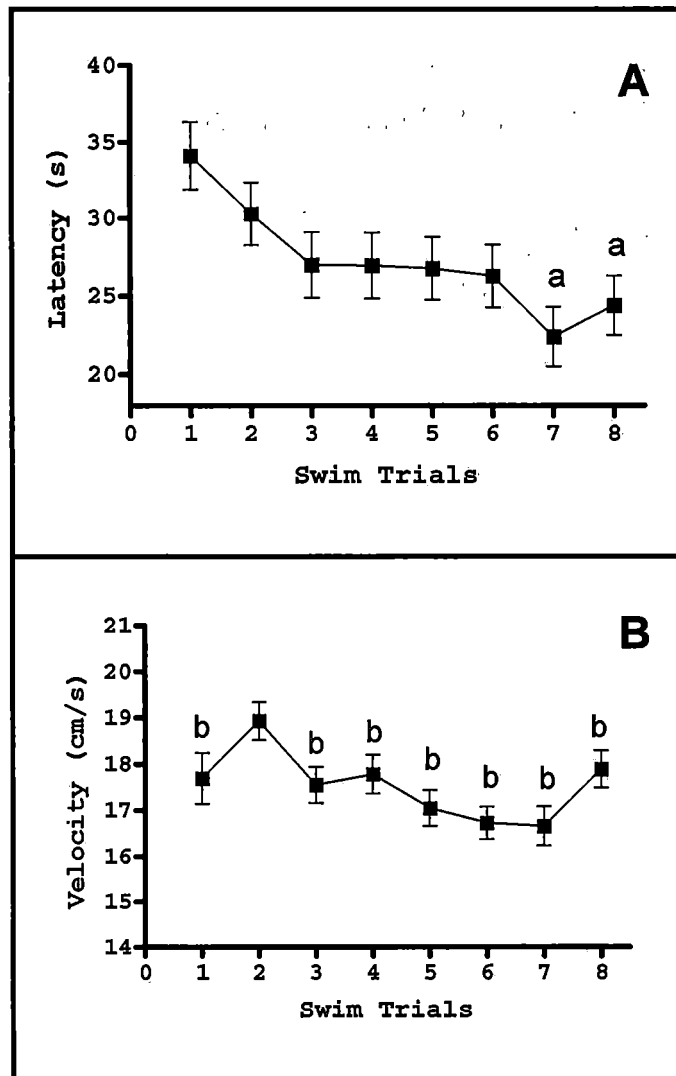


Figure 9. Mean Latency (s) (Panel A) and Swim Velocity (cm/s) (Panel B) (\pm SEM) to Locate Escape Platform Across the Eight Training Trials on the MWM for All Mice on the Conditioning Day (Day 6). ^aSignificantly Different from Swim Trials 1 and 2 ($P < 0.05$). ^bSignificantly Different from Swim Trial 2 ($P < 0.05$)

To ensure that no differences between the groups existed by the end of the conditioning day, and that all subjects performed similarly prior to drug injection, additional one-way ANOVAs were conducted on trial 8 (Gresack & Frick, 2006). Indeed, all subjects performed similarly on trial 8 as indicated by a non-significant drug main effect for latency ($F_{4,102} = 1.19, P > 0.05$) or velocity ($F_{4,102} = 0.40, P > 0.05$). Also, a separate one-way ANOVA with sex as the independent variable indicated that males and females located the platform similarly prior to drug injection (latency: $F_{1,105} = 2.32, P > 0.05$). On the other hand, swim velocities were slightly, but significantly, faster for males than females on trial 8 ($F_{1,105} = 4.32, P < 0.04$).

In summary, data from the conditioning day indicated that all mice were performing similarly prior to drug injection and did not differ as a function of group (drug) assignment or sex. Because sex differences were not detected throughout spatial memory training (conditioning day) all Test Day analyses were collapsed across sex.

Test Day 1

The effects of posttraining injections of cocaine on spatial memory retention are displayed in Figure 10. In contrast to trial 8 on the conditioning day (last trial prior to drug injection), there were significant cocaine-induced differences on Test Day 1. Specifically, mice administered 2.5 mg/kg cocaine found the escape platform in significantly less time when compared to saline controls (drug main effect: $F_{4,102} = 3.90$, $P < 0.05$, and Tukey tests). Furthermore, this same group (cocaine 2.5 mg/kg) also found the escape platform in significantly less time than the groups administered 5.0 or 20.0 mg/kg of cocaine, but not the group injected with 1.5 mg/kg cocaine (Tukey tests). Importantly, motor behavior (swim velocity) was not affected by cocaine on Test Day 1 (24 hr post injection) ($F_{4,102} = 1.01$, $P > 0.05$) (see Figure 10B). Together, these data indicated that water maze performance on Test Day 1 was due to cocaine's effects on spatial memory retention and not a result of drug induced changes in motor ability.

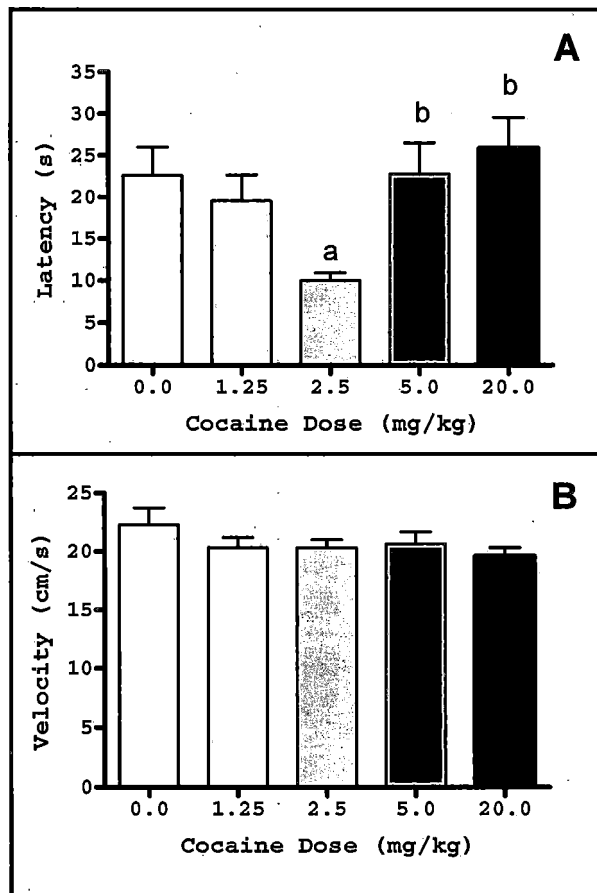


Figure 10. Mean Latency (s) (Panel A) and Swim Velocity (cm/s) (Panel B) (\pm SEM) to Locate Escape Platform on Test Day 1 in C57 Mice 24 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg). ^aSignificantly Different from Saline Controls ($P < 0.05$). ^bSignificantly Different from Cocaine 2.5 mg/kg Group ($P < 0.05$)

Test Day 2

Cocaine did not alter water maze performance when tested 48 hr after drug injection, because no differences in latency (see Figure 11A) or velocity (see Figure 11B) were found. This suggested that the effects of cocaine on spatial memory consolidation did not persist 48 hr after drug injection.

Body Weights

Across behavioral testing, males weighed significantly more ($\bar{X} = 23.14$ mg) than females ($\bar{X} = 19.56$ mg) regardless of group assignment on the conditioning day ($F_{1,105} = 125.42$, $P < 0.001$), Test Day 1 ($F_{1,105} = 126.88$, $P < 0.001$), and Test Day 2 ($F_{1,48} = 51.74$, $P < 0.001$).

Separate one-way ANOVAs on the conditioning day, with drug group as the independent variable, indicated that body weights did not differ according to drug-group assignment ($F_{4,102} = 0.14$, $P > 0.05$). Cocaine administration also did not affect body weight 24 hr (Test Day 1, $F_{4,102} = 0.14$, $P > 0.05$) or 48 hr (Test Day 2, $F_{4,45} = 0.52$, $P > 0.05$) after

cocaine injection. Importantly, this indicates that changes in water maze performance were not due to changes in body weight.

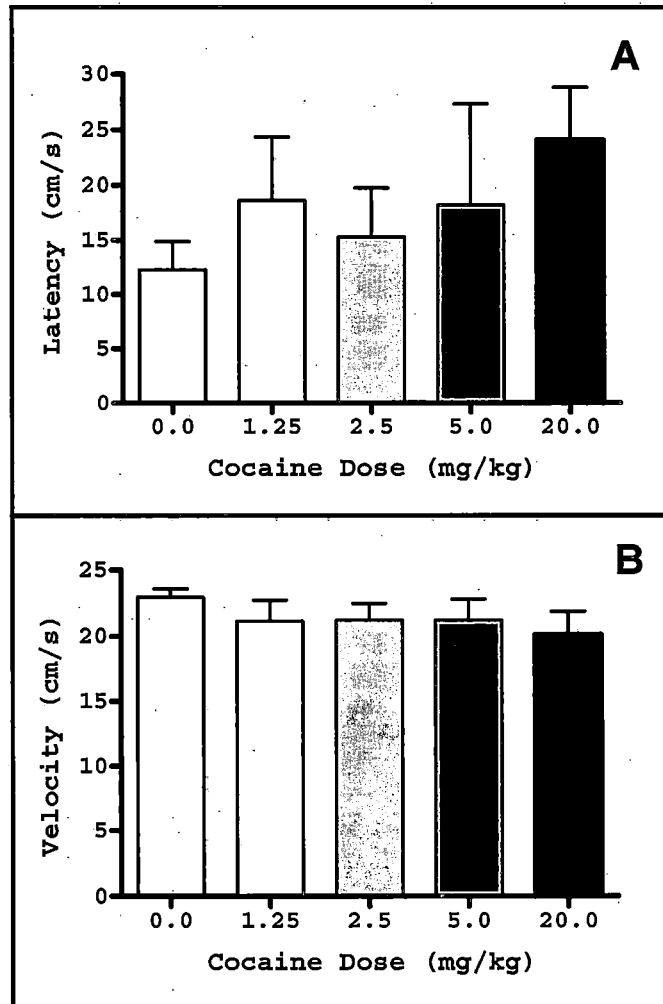


Figure 11. Mean Latency (s) (Panel A) and Swim Velocity (cm/s) (Panel B) (\pm SEM) to Locate Escape Platform on Test Day 2 in C57 Mice 48 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg)

Table 2. Body Weights

Test-Day	Cocaine Treatment (mg/kg)				
	0.0	1.25	2.50	5.00	20.00
Conditioning	21.00 (±0.49)	21.26 (±0.53)	20.86 (±0.51)	21.38 (±0.62)	21.24 (±0.58)
Test-Day 1	21.21 (±0.50)	21.45 (±0.54)	21.04 (±0.48)	21.57 (±0.60)	21.35 (±0.55)
Test-Day 2	21.55 (±0.77)	21.55 (±0.73)	20.86 (±0.87)	21.81 (±0.91)	21.18 (±0.68)

Numbers in parenthesis indicate standard error of the mean (\pm SEM). Conditioning Day indicates body weights prior to drug injection. Test-Day 1 indicates body weights 24 hours post drug injection. Test-Day 2 indicates body weights 48 hours post drug injection.

Hippocampal PKA Activity

Bilateral hippocampal tissue was extracted immediately after behavioral testing in order to assess PKA activity. Because mice administered 1.25 mg/kg cocaine did not differ behaviorally from any other group on Test Day 1 (see Figure 10A), it was not included in the analysis.

When assayed 24 hr after drug administration, there was a significant difference in hippocampal PKA activity between male ($n = 20$) and female ($n = 28$) C57 mice (sex main effect: $F_{1,40} = 15.12, P < 0.05$). Specifically, on Test Day 1, regardless of drug group, female mice exhibited

higher levels of hippocampal PKA activity (3.64 ± 0.19 nmol/min/mg protein) than male mice (2.48 ± 0.22 nmol/min/mg protein) (see Figure 12).

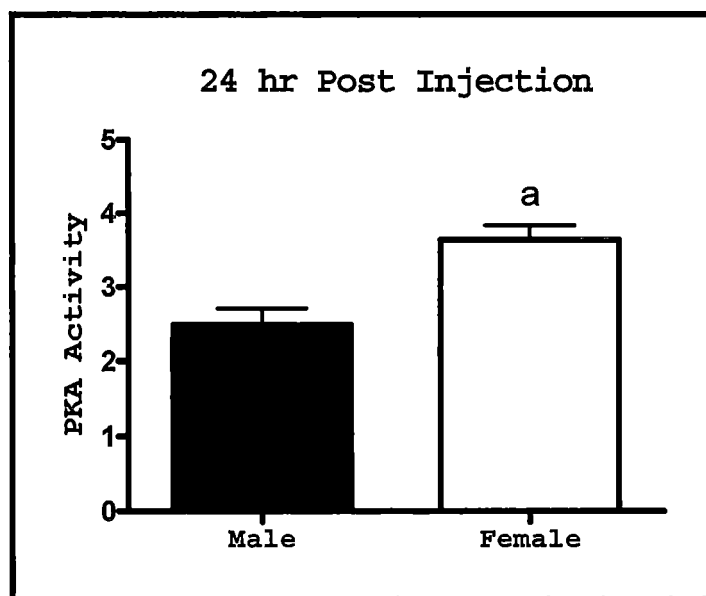


Figure 12. Mean Hippocampal PKA Activity (nmol/min/mg protein) in C57 Male and Female Mice on Test Day 1, 24 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg). ^aSignificantly Different from Male Mice ($P < 0.05$)

When data from female mice were analyzed separately, a significant drug main effect ($F_{3,28} = 5.06, P < 0.05$) was found involving hippocampal PKA activity on Test Day 1 (see Figure 13). Specifically, when compared to saline controls, female mice administered 2.5 or 20.0 mg/kg displayed higher levels of hippocampal PKA activity when compared to controls (Dunnetts, $P < 0.05$). Although 5.0 mg/kg cocaine also increased hippocampal PKA activity in female mice, this difference did not reach statistical significance (Dunnetts, $P > 0.05$). Male mice did not exhibit a similar dose-dependent increase of PKA activity (see Figure 14).

Cocaine-induced differences in hippocampal PKA activity were not detected 48 hr after drug injection (Test Day 2; see Figure 15).

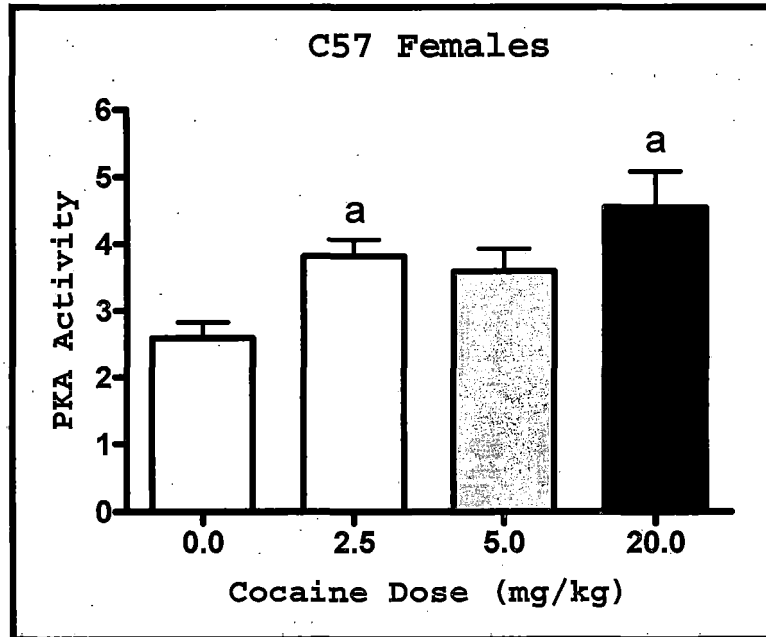


Figure 13. Mean Hippocampal PKA Activity (nmol/min/mg protein) in Female C57 Mice on Test Day 1, 24 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg). ^aSignificantly Different from Saline Control Group ($P < 0.05$)

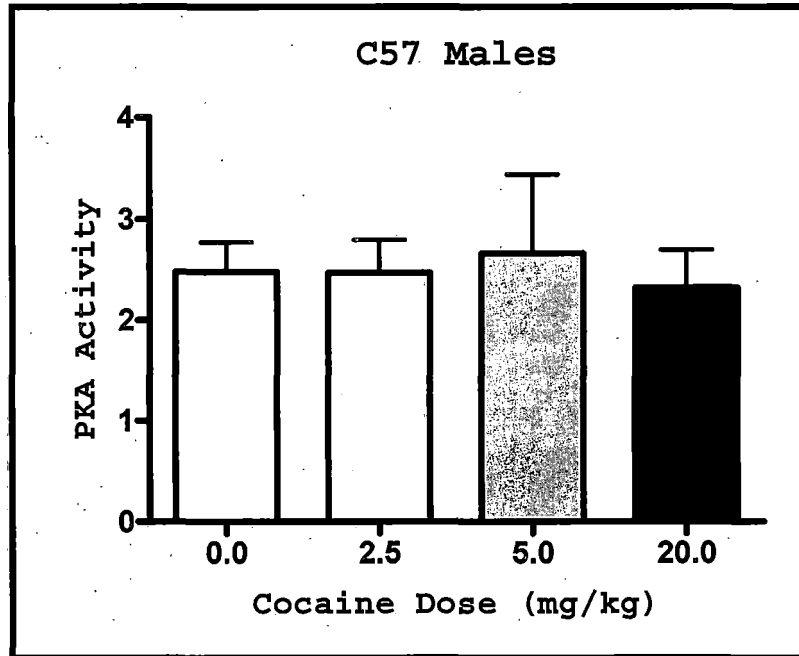


Figure 14. Mean Hippocampal PKA Activity (nmol/min/mg protein) in Male C57 Mice on Test Day 1, 24 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg)

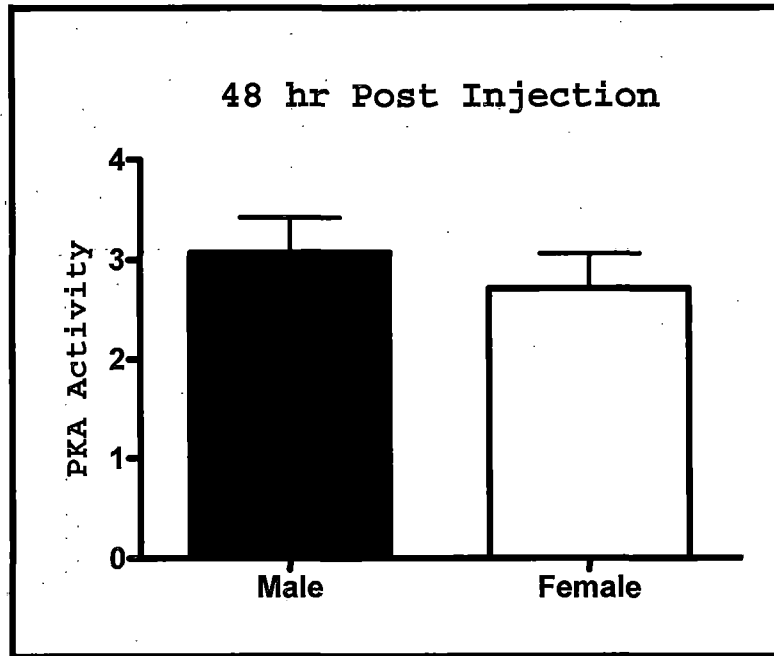


Figure 15. Mean Hippocampal PKA Activity (nmol/min/mg protein) in Male and Female C57 Mice on Test Day 2, 48 hr After Eight Training Trials on the MWM and Injected with Cocaine (0.0, 1.25, 2.5, 5.0, or 20.0 mg/kg)

CHAPTER TEN

DISCUSSION

Effect of Acute Cocaine Administration on Spatial Memory

The goal of the present investigation was to determine if an acute posttraining injection of cocaine would facilitate spatial memory performance in C57 mice. To this end, male and female mice were trained on a MWM spatial memory task, injected with saline or cocaine (1.25, 2.5, 5.0, or 20.0 mg/kg), and then tested after a 24 and/or 48 hr delay (see Table 1). This behavioral protocol (posttraining injections) was adopted in order to avoid any possible confounding effects of pre-training drug administration on test performance (Gresack & Frick, 2006).

Because of cocaine's ability to increase synaptic dopaminergic levels, it was hypothesized that cocaine would affect spatial memory performance on the MWM as a function of drug dose. Specifically, it was hypothesized that low doses of cocaine would facilitate spatial performance (1.25 mg/kg for female mice; 2.5 and 5.0 for male mice), while high doses would impair it (20.0 mg/kg in both male and female mice).

The results of the present study indicated that both male and female C57 mice performed similarly on the MWM. Specifically, there were no differences in latencies (time to find the escape platform) or swim velocities between male and female mice. This result was surprising since a large body of literature suggests that males usually perform better than females on spatial tasks (for a review see Lawton & Morrin, 1999; but see Eals & Silverman, 1997; Heale & Harley, 1990; Healy, Braham, & Braithwaite, 1999). One possible explanation for this inconsistency is that the spatial task used in the current investigation was not sufficiently complex to promote sex differences.

Consistent with this interpretation, Coluccia and Louse (2004) have suggested that sex differences involving spatial ability only occur when the task is very difficult.

On Test Day 1 (24 hr post injection), cocaine had the predicted effect of improving spatial memory performance on the MWM (time to locate the platform; see Figure 10A). Yet, the hypothesis that different doses of cocaine would facilitate spatial performance between male and female mice was not supported (1.25 mg/kg for female mice; 2.5 and 5.0 for male mice). Interestingly, the optimal dose of cocaine to facilitate spatial ability (in both male and female

mice) was 2.5 mg/kg. Overall, cocaine's effects on spatial ability were represented by a U-shape function where both the lowest (1.25 mg/kg) and highest (5.0 and 20.0 mg/kg) doses did not affect MWM performance, while only the 2.5 mg/kg dose significantly enhanced performance. The present results are in agreement with previous reports where 2.5 mg/kg cocaine facilitated memory performance on a shock avoidance memory task (Castellano et al., 1996). When considered together, cocaine is able to enhance memory performance on both simple avoidance paradigms as well as in spatial memory tasks using the MWM. Surprisingly, the prediction that a high dose of cocaine (20.0 mg/kg) would impair spatial memory performance on the MWM was not supported. Although mice administered the greatest dose of cocaine (20.0 mg/kg) had the longest latencies on Test Day 1, this group did not statistically differ from saline controls. Previous investigations have reported that high doses of cocaine (20-40 mg/kg) administered chronically prior to training impaired memory consolidation on a MWM task (Quirk et al., 2001). Yet, in the present study administering 20 mg/kg cocaine did not impair spatial memory performance on the MWM. These inconsistent results can probably be attributed to differences in experimental

design. In the present study, cocaine was administered after training and not while the subject was learning the task. Since memory is more susceptible to changes or modifications after the completion of the task (see Gresack & Frick, 2006; Janak et al., 1992), it is likely that the timing of cocaine administration was responsible for differences between studies. Also, another explanation could be that a 20 mg/kg dose of cocaine may not be high enough to induce memory impairment, as previous investigations using rats have found 15-20 mg/kg doses of cocaine to facilitate operant conditioning memory tasks (Taylor & Jentsch, 2001; White et al., 1995). Considering these investigations together, where 15-20 mg/kg cocaine facilitated memory performance and 20-40 mg/kg impaired it, it is possible that the greatest dose used in this study (20.0 mg/kg) was simply not high enough to induce memory impairment, as it was marginally close to those previously found to facilitate performance in rats (Taylor & Jentsch, 2001; White et al., 1995).

Cocaine did not affect spatial memory performance in C57 mice when tested 48 hr post drug administration (Test Day 2). Indeed, neither cocaine nor sex affected latency or swim velocity to reach the escape platform 48 hr post

injection (see Figure 11A). The results suggest that the enhancing effects of cocaine on spatial memory are time dependent and do not carry over beyond 48 hours.

Overall, the behavioral data in this study provides support to the theory that low doses of cocaine enhance spatial memory consolidation in rodents (Ciamei et al., 2000; Introini-Collison & McGaugh, 1989; Janak et al., 1992). Because cocaine was administered after training (posttraining injections), and no differences in swim velocity (see Figures 10B and 11B) or body weight (see Table 2) were detected across the groups, it is reasonable to suggest that the effects of cocaine on MWM performance were the result of enhanced spatial memory consolidation and not physical effects induced by cocaine (i.e., swimming faster thus finding the platform faster or by losing body weight). Lastly, it is important to note that although cocaine was administered immediately after training (a delayed-injection group was not used in the study), other studies have shown that injecting cocaine one hour (Introini-Collison & McGaugh, 1989) and/or two hours (Castellano et al., 1996) after training does not affect memory consolidation. Thus, it is likely that the effects

of cocaine on spatial memory are likely to be limited to the one- and two-hour period immediately after injection.

Effect of Acute Cocaine Administration on Hippocampal PKA Activity

Because the hippocampus is important for spatial memory (see Chapter 2), and recent investigations have suggested that dopamine modulates memory consolidation in the hippocampus via cAMP-dependent PKA systems (Izquierdo, Barros, Ardenghi, Pereira, Rodrigues, Choi, Medina, & Izquierdo, 2000; Yamamoto, Urakubo, Tominaga-Yoshino, & Ogura, 2005), hippocampal PKA was assayed in mice after behavioral testing. It was hypothesized that cocaine administration (2.5, 5.0, and 20.0 mg/kg) would increase hippocampal PKA activity (when compared to saline controls) in both male and female C57 mice tested on the MWM. Furthermore, it was expected that the group displaying enhanced behavioral spatial memory performance on the MWM would also display the highest hippocampal PKA.

Interestingly, the hypothesis that cocaine would increase PKA in the hippocampus of male and female C57 mice was only partially supported. A sex difference in PKA activity was found on Test Day 1, in which female mice

displayed higher hippocampal PKA activity than males (see Figure 13). Within male mice, no cocaine-induced differences in PKA activity were observed (Figure 15). In contrast, cocaine dose-dependently increased the amount of PKA in the hippocampus of female mice (see Figure 14). This increase in PKA activity may explain the ability of cocaine to enhance memory performance, given that past research suggests that PKA modulates spatial memory consolidation processes in the hippocampus (see Figure 5; Mizuno, Yamada, Maekawa, Saito, Seishima, & Nabeshima, 2002; Sibley & Monsma, 1992). In the present study, female mice given 2.5 mg/kg cocaine displayed both behaviorally enhanced spatial memory performance and significantly higher levels of PKA (in comparison to controls). This conclusion should be tempered however, since (a) the same pattern of results was not observed in male mice and (b) female mice administered 20.0 mg/kg cocaine displayed enhanced PKA activity while not displaying enhanced spatial memory performance. Thus, spatial performance (on the MWM) and hippocampal PKA appear to be dissociated. Based on this result, it is possible that PKA may not be at the level necessary to assess spatial performance.

Furthermore, the most current research on the biological aspects of learning and memory suggests that CREB phosphorylation (which leads to gene transcription and memory consolidation) can be mediated via protein kinases other than PKA, including protein kinase C (PKC), tyrosine kinase Fyn, MAPK, and type two calcium calmodulin-dependent protein kinase (CaMKII) (Hinoi et al., 2002). Therefore, it is possible that other protein kinases may be indirectly activated by dopamine and may be responsible for spatial memory enhancement in C57 mice when tested on the MWM. Clearly, further neurochemical research is needed to better understand the role of protein kinases in cocaine-induced spatial memory consolidation in C57 mice when tested on the MWM.

The finding that female mice displayed greater hippocampal PKA activity than male mice on Test Day 1 suggests that females are more sensitive to cocaine than males (see also Festa et al., 2004). Interestingly, few studies have reported a difference in PKA activity as a function of sex. As a matter of fact, most investigations reporting sex differences in PKA activity have done so only when rodents have been pretreated with psychostimulant drugs during the preweanling period and then tested as

adults (Crawford, Williams, Kohutck, Choi, Yoshida, McDougall, & Vorhees, 2006; Lynch, Kiraly, Caldarone, Picciotto, & Taylor, 2006). In this circumstance, females also exhibit greater PKA activity than males.

The reason why differences in hippocampal PKA activity exist between male and female mice is not known. A possible explanation for the enhanced sensitivity to cocaine in female hippocampus is that estrogen may play a neuroprotective role when female subjects are administered a psychostimulant drug (see Gao & Dluzen, 2001; Morissette, Jourdain, Sweidi, Menniti, Ramirez, & Paolo, 2007). Or, perhaps a higher concentration of G_s receptors are found in the postsynaptic terminals of hippocampal dopaminergic neurons in female rodents when compared to males, since estrogen up-regulates dopamine D_1 receptor gene transcription factors (see Lee & Mouradian, 1999). In either case, further molecular research is required to fully understand the biological bases of increased female sensitivity to cocaine when compared to males, with special attention given to measuring mesolimbic D_1 (G_s) receptor levels.

No differences in hippocampal PKA activity 48 hr post cocaine administration (Test Day 2) were detected. Similar

to the behavioral data, the effects of cocaine on hippocampal PKA activity (see Figure 15) was only observed 24 hr post drug injection, thus suggesting that the effects of cocaine on hippocampal PKA activity (in female C57 mice) are transient.

Conclusion

The present investigation demonstrates for the first time that posttraining injections of cocaine (2.5 mg/kg) can significantly facilitate spatial memory consolidation in male and female C57 mice. The fact that posttraining cocaine administration allowed memory to be examined in the absence of drug-induced confounds related to task performance (i.e., physical effects of cocaine on motor ability and/or body-weight loss) suggested that dopamine may selectively enhance spatial memory consolidation. Because hippocampal PKA activity did not correlate with MWM performance, the mechanism by which dopamine facilitated spatial memory consolidation may not directly require PKA activation.

Although the underlying mechanisms of cocaine-induced memory enhancement are still not clearly defined, this study provides new information about dopamine's ability to facilitate memory consolidation processes of spatial

memory. The results of this investigation as well as results from previous studies using simple avoidance memory tasks, together suggest that increasing dopamine levels in the brain immediately after learning a task can facilitate both non-declarative and declarative memory consolidation. As such, this information may have important clinical implications for the development of cognitive enhancers. Specifically, scientists interested in memory dysfunction, impairment, and/or improvement, can use this information to develop novel drugs that aim to selectively increase dopamine levels in the brain (to facilitate memory consolidation processes) and enhance memory performance.

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