Title
Formation and Maintenance of Appetitive Pavlovian Associations

Author
Blaiss, Cory

Publication Date
2007-08-31

Peer reviewed|Thesis/dissertation

Formation and Maintenance of Appetitive Pavlovian Associations

by

Cory A. Blaiss

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Acknowledgements

The completion of this dissertation would not have been possible without the help of a great many people. I feel incredibly lucky to know such clever, creative, funny, warm, and caring individuals, and while I do not have enough space in these pages to thank them all by name, they have all made a difference in my life and career.

I am enormously grateful to my advisor, Tricia Janak, for all that she has taught me. A student's dissertation advisor has a singular impact on the quality of the graduate school experience, and Tricia's talent and grace ensured that my experience was enjoyable as well as edifying. Tricia has been and continues to be a superb mentor. She genuinely cares about the interests and career development of her lab members, she is extremely knowledgeable about her field, and she is an open, honest, ethical, inquisitive, and thoughtful scientist. In addition to being a wonderful scientist, Tricia is also a wonderful person, and I have a huge amount of respect for the balance that she has achieved in her life.

I have also been fortunate to have a tremendous thesis committee. Bob Messing, Steve Lisberger, Michael Brainard, and Bernard Balleine have all been extremely generous with their time, and they each have a knack for offering excellent advice with perfect timing. I would also like to thank Howard Fields for being an additional mentor to me throughout my graduate career and constantly inspiring me to think about the big picture questions in science.

The Janak lab is a fantastic group of people, and many thanks go to all the past and present members of the lab for collectively creating such an amazing atmosphere in

which to do science. I would like to thank them for all the advice, the technical help, the late night discussions of science, the excellent Wine Hours, and most importantly, their friendship. I would especially like to thank Lacey Sahuque for scoring so much freezing behavior for the experiments described in Chapter Four, Margaret Tsiang Kirkham for getting me comfortably settled in when I joined the lab, and Mike Gill for patiently and humorously answering my constant barrages of questions and teaching me how to be a detailed scientist.

The Gallo Center is full of excellent scientists and support staff who helped me in numerous aspects of my research. Rajana Van Bautista provided technical assistance with some of the histology for the experiments described in Chapter Four, and Joe Kim was a wonderful guide to the world of conditioned place preference when I was designing the experiments described in Chapter Two. Frank Ponce and the rest of the animal facility staff went above and beyond the call of duty to make sure that my rats were healthy and happy. Michael Whitman patiently explained many fundamentals of research grants and helped me to obtain one (many thanks go to the National Institute on Drug Abuse at the National Institutes of Health for supporting me and my research). Dick Dixon, Calvin Hom, and Brandon Abrams, Sr. helped me with countless computer malfunctions and let me hang around their office when I needed a break from my research. I have also been fortunate to make some wonderful friends at the Gallo, and I would like to thank them for their continued support (and soccer and wine and dinners).

The UCSF Neuroscience Graduate Program is such a collegial and collaborative community, and I would like to thank Lou Reichardt, the program director, for being such a tireless advocate for students. I learned so much from him when I was a student

representative for the program, and it was a pleasure to work with him. Pat Veitch has been a superb guide through the bureaucracies of graduate school, and I appreciate all the work that she has done to ensure that I encountered a minimum of logistical obstacles.

I am also very grateful to my neuroscience classmates: Sarah Bauer Huang, Matt Caywood, John Proctor, Ammon Corl, and Kaiwen Kam. They are all curious and talented scientists as well as incredibly kind, caring, and tolerant people, and they have all become lifelong friends. I would especially like to thank Sarah; I am so appreciative of her constant support, and her centered perspective on life is always refreshing. I would also like to thank Ritu Kapur for her humor, her generosity, and her amazing passion for learning. I am lucky to have such a wonderful friend, and I could not imagine graduate school without her.

I am also lucky to have an incredible support system of family and friends outside of science. Many close friends effectively became members of my extended family when I was very young. They have given me a lifetime of encouragement and love, and they have always ensured that my life includes joy, laughter, and balance. I would especially like to thank John Evans, who has given me unwavering and unquestioning support and whose warmth, caring, and openness always offer the most wonderful comfort for any of life's stresses.

I would also like to thank my parents, Ann Blaiss and Gary Blaiss, who have given me unconditional love, support, and encouragement throughout my life. My dad has always shared his love of science and math with me. When I was young, he taught me about concepts that I didn't encounter in school until years later, and he has always made concepts about astronomy, medicine, and math seem wondrous and exciting. My

mom taught me how to live, and I would have become a completely different person without her wisdom, insight, and guidance.

Preface

Parts of this dissertation have been previously published elsewhere. Chapter Two is a reprint of the material as it appears in *Behavioural Brain Research* [C.A. Blaiss and P.H. Janak, "Post-training and post-reactivation administration of amphetamine enhances morphine conditioned place preference",Volume 171, Issue 2, Pages 329-227, Copyright (2006)]. Chapter Three is a reprint of the material as it appears in *Neurobiology of Learning and Memory* [C.A. Blaiss and P.H. Janak, "Post-training, but not post-reactivation, administration of amphetamine and anisomycin modulates Pavlovian conditioned approach", Volume 87, Issue 4, Pages 644-658, Copyright (2007)]. The co-author listed in these publications directed and supervised the research that forms the basis for this dissertation. This material is reprinted with permission from Elsevier (see below for permission).

Blaiss, Cory

From: Moss, Marion (ELS-OXF) [m.moss@elsevier.co.uk] **Sent:** Tue 4/10/2007 3:35 AM
To: Blaiss, Cory
Cc:
Subject: RE: Obtain Permission
Attachments:

ELSEVIER

Dear Cory Blaiss

We hereby grant you permission to reproduce the material detailed below at no charge in your thesis subject to th following conditions:

1. If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not then that material may not be included in your publication/copies.

2. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of publication, as follows:

"Reprinted from Publication title, Vol number, Author(s), Title of article, Pages No., Copyright (Year), with permiss Elsevier".

3. Your thesis may be submitted to your institution in either print or electronic form.

4. Reproduction of this material is confined to the purpose for which permission is hereby given.

5. This permission is granted for non-exclusive English rights only. For other languages please reapply separatel each one required.

6. This includes permission for UMI to supply single copies, on demand, of the complete thesis. Should your thes published commercially, please reapply for permission.

Yours sincerely

Marion Moss
Senior Rights Assistant
Elsevier Ltd
The Boulevard
Langford Lane
Kidlington
Oxford
OX5 1GB

Tel : +44 1865 843280
Fax : +44 1865 853333
E-mail : m.moss@elsevier.com

This Email was sent from the Elsevier Corporate Web Site and is related to Obtain Permission form:

Product: Customer Support
Component: Obtain Permission
Web server: http://www.elsevier.com
IP address: 10.10.24.148
Client: Mozilla/5.0 (Windows; U; Windows NT 5.1; en-US; rv:1.8.0.11) Gecko/20070312 Firefox/1.5.0.11
Invoked from: http://www.elsevier.com/wps/find/obtainpermissionform.cws_home?isSubmitted=yes&navigateXmlFileName=/store/prod_webcache_act/framework_support/obtainpermission.xml

Request From:
Cory Blaiss
University of California, San Francisco
5858 Horton St., Suite 200
94608
Emeryville
United States

Contact Details:
Telephone: 510-985-3897
Fax: 510-985-3101
Email Address: cory.blaiss@ucsf.edu

To use the following material:
ISSN/ISBN:
Title: Neurobiology of Learning and Memory
Author(s): Cory A. Blaiss and Patricia H. Janak
Volume: 87
Issue: 4
Year: 2007
Pages: 644 - 658
Article title: Post-training, but not post-reactivation...

How much of the requested material is to be used:
I would like to use the entire article mentioned above. In addition, I would like to use another Elsevier journal arti entirety. The citation for the second article is in the last field of this page.

Are you the author: Yes
Author at institute: Yes

How/where will the requested material be used: [how_used]

Details:

I would like to use these two journal articles as separate chapters in my dissertation at the University of California Francisco.

Additional Info: I would like to see if I could obtain written permission to use both of these journal articles for n dissertation. I will need to include a letter with this permission in the final copy of my dissertation. The citation fo second journal article is below:

Blaiss, Cory A. and Patricia H. Janak (2006) "Post-training and post-reactivation administration of amphetamine e morphine conditioned place preference". Behavioural Brain Research, 171(2): 329-227.

Thank you for your help.

- end -

For further info regarding this automatic email, please contact:
WEB APPLICATIONS TEAM (esweb.admin@elsevier.co.uk)

Abstract

Formation and Maintenance of Appetitive Pavlovian Associations

by Cory A. Blaiss

Environmental stimuli associated with a drug or natural reward have the ability to elicit strong cravings and relapse bouts in human addicts and individuals with addiction-related psychopathologies such as compulsive eating disorders. In order for these environmental stimuli to drive reward-seeking behavior, however, Pavlovian associations between the environmental stimuli and the reward must first be acquired, and the memory of those associations must be successfully maintained. This aim of this dissertation is to investigate the mechanisms underlying the formation and maintenance of such appetitive Pavlovian associations, using two models of appetitive Pavlovian conditioning: morphine conditioned place preference (mCPP) and Pavlovian conditioned approach (PCA).

The first set of experiments described in this dissertation investigated the ability of the psychostimulant amphetamine (AMPH) to modulate the consolidation and reconsolidation of mCPP. These experiments found that AMPH injected immediately after training and memory reactivation enhanced mCPP. The second set of experiments investigated the effect of AMPH and the protein synthesis inhibitor anisomycin (ANI) on the consolidation and reconsolidation of PCA. Both AMPH and ANI modulated PCA behavior when injected immediately after training, but not when injected after memory reactivation. The third set of experiments used reversible inactivation as well as infusions of ANI to investigate the role of the nucleus accumbens (NAcc) core and shell

in the consolidation and expression of PCA and cued fear conditioning, a form of aversive Pavlovian conditioning. These experiments showed that the NAcc core (but not the shell) is involved in the consolidation, but not the expression, of cued fear conditioning. In contrast, both NAcc subnuclei were necessary for the expression, but not the consolidation, of PCA.

These findings collectively demonstrate that the consolidation of mCPP and PCA can be modulated in a manner similar to other types of learning and memory, that a drug of abuse such as AMPH can positively modulate the consolidation of addiction-related learning, and that there are differences in the degree of post-reactivation lability exhibited by different forms of appetitive Pavlovian conditioning. These findings also demonstrate a surprising dissociation between the role of the NAcc in appetitive and aversive Pavlovian conditioning.

Michael S. Brainard, Committee Chair

6/8/07

Date

Table of Contents

List of Tables and Figures

Chapter One:
General Introduction

Environmental stimuli associated with a drug or natural reward have the ability to elicit strong cravings and relapse bouts in human addicts and individuals with addiction-related psychopathologies such as compulsive eating disorders (Heather et al., 1991; Rohsenow et al., 1994; Jansen, 1998; Sobik et al., 2005). In order for these environmental stimuli to drive reward-seeking behavior, however, the Pavlovian associations between the environmental stimuli and the reward must first be acquired, and the memory of those associations must be successfully maintained. This aim of this dissertation is to investigate the mechanisms underlying the formation and maintenance of such appetitive Pavlovian associations, using two models of appetitive Pavlovian conditioning: morphine conditioned place preference (mCPP) and Pavlovian conditioned approach (PCA). In this chapter, I will discuss the rationale for this line of investigation and provide background information for the chapters that will follow.

The Effect of Environmental Stimuli on Relapse

Drug addiction and related disorders of compulsive behavior are a large social problem in the United States and worldwide. In 2003, approximately 4.7% of people in the world over age 15 were abusing drugs or alcohol (United Nations Office on Drugs and Crime, 2003); between 1990 and 1992, approximately 24.1% of the U.S. population aged 15-54 was dependent on tobacco, 14.1% of the population was dependent on alcohol, and 7.5%

of the population was dependent on other illicit drugs (Anthony et al., 1994). Treating addiction can also have a large cost; in fiscal year 2006, the United States government spent 4.8 million dollars on the prevention and treatment of addiction and addiction-related disorders (Office of National Drug Control Policy, 2007).

One of the major problems undermining the ability of clinicians to successfully treat these disorders is the high rate of relapse among addicts and individuals with addiction-related psychopathologies. Even with treatment, a large percentage of addicts and alcoholics eventually relapse (Stapleton, 1998; McKay and Weiss, 2001; Witkiewitz and Marlatt, 2004). For example, even when smokers were treated with the nicotine patch, 60-80% of subjects had resumed smoking 6 months after the start of treatment (Stapleton, 1998), and because of results such as this, one researcher concluded that "relapse is the most likely outcome of any smoking cessation attempt" (Piasecki, 2006). Although relapse has not been as well studied in addiction-related disorders, patients with addiction-related psychopathologies such binge-eating disorder and pathological gambling do show high rates of relapse (Agras, 2001; Ledgerwood and Petry, 2006).

Environmental stimuli associated with drugs play a critical role in the problem of relapse because of their ability to induce relapse bouts and strong feeling of craving in addicts. Cocaine users report increased feelings of drug craving after exposure to cocaine-associated cues compared to cues that have not been associated with cocaine (Childress et al., 1999; Garavan et al., 2000). Cue-induced cravings have been shown to induce relapse bouts in heroin addicts and alcoholics (Heather et al., 1991; Rohsenow et al., 1994), and certain food-related cues can induce bouts of overeating in binge eaters (Jansen, 1998; Sobik et al., 2005). This ability of environmental stimuli to induce relapse

has also been modeled in animals in the context- and cue-induced reinstatement tasks. Re-exposure to either a context or a discrete cue previously associated with the delivery of a number of different rewards can reinstate reward-seeking behavior in abstinent rats (Meil and See, 1997; Grimm and See, 2000; See et al., 2001; Nie and Janak, 2003; Zironi et al., 2006).

Multiple laboratories have focused on the problem of relapse through the study of the neural circuitry and pharmacology underlying such context- and cue-induced reinstatement tasks (See, 2002; Bossert et al., 2006; Burattini et al., 2006). However, few have approached this problem as purely a learning and memory question. Ultimately, though, without an intact memory trace and the ability to repeatedly recall the association between the environmental stimuli and the drug reward, the environmental stimuli could not have a strong impact on an addict's propensity to relapse. Pavlovian associations between the environmental stimuli and the reward must be acquired at some point in the development of an addiction, and the memory of those associations must be maintained in order for the stimuli to continue to drive reward-seeking behavior in addicts and in individuals with addiction-related disorders such as compulsive eating disorders. An understanding of how these appetitive Pavlovian associations are formed and maintained could eventually provide a novel treatment approach for relapse prevention. Therefore, the studies in this dissertation have investigated the mechanisms underlying the formation and maintenance of two different forms of appetitive Pavlovian conditioning.

Pavlovian Conditioning

As the name implies, appetitive Pavlovian (or classical) conditioning was first described by Ivan Pavlov in his Nobel Prize winning studies with dogs (Pavlov, 1927). In his study of salivary glands, Pavlov had shown that the amount of saliva produced by a dog increased when a bit of meat powder was put on the dog's tongue. During the course of his experiments, he observed that after an auditory stimulus was presented contingently with the placement of the meat powder on the dog's tongue, the auditory stimulus alone became sufficient to elicit the increase in salivation (Pavlov, 1927). In general, Pavlovian conditioning tasks involve associational learning between an initially neural stimulus (the conditioned stimulus, CS) and another stimulus (the unconditioned stimulus, US) that elicits an innate physiological response (unconditioned response, UR), like salivation in the case of Pavlov's dogs. When the CS is presented contingently with the US, presentation of the CS alone will come to elicit a learned physiological response known as the conditioned response (CR) (Rescorla and Wagner, 1972). This dissertation focuses mainly on two varieties of appetitive Pavlovian conditioning: morphine conditioned place preference (mCPP) and Pavlovian conditioned approach (PCA).

In the mCPP task, a distinct environmental context is associated with the administration of morphine. On a series of days, rats are injected with morphine and confined for a limited time in a distinct environment. On alternate days, rats are injected with saline and confined for a limited time in a separate distinct environment. On a test day, rats are allowed to explore both environments, and rats that have learned to associate the rewarding effects of morphine (the US) with the morphine-paired environment (the

CS) spend more time in the morphine-paired environment (Rossi and Reid, 1976; Bardo and Bevins, 2000). In addition to morphine, animals can also develop conditioned place preferences when the environmental context is associated with a number of other drug rewards, such as cocaine and LSD, as well as natural rewards, such as home cage odors and social interaction (Tzschentke, 1998).

Since it was first described in 1976, most experiments investigating the physiology and pharmacology of mCPP have been designed to examine the neurobiology of the motivational aspects of the task, namely the rewarding properties of morphine (Rossi and Reid, 1976; Tzschentke, 1998). Although there is little known about the mechanisms underlying the formation and maintenance of the Pavlovian associations involved in the mCPP task, some studies have specifically addressed this issue. Lesions of the periacqueductal gray (PAG) and the fornix impair the acquisition of mCPP, and because the animals are still sensitive to the rewarding effects of morphine, it has been hypothesized that these nuclei are critical to the associational learning of the task (Olmstead and Franklin, 1997). Studies investigating molecular mechanisms of mCPP acquisition have found that inhibition of protein synthesis as well as the kinase ERK prevents learning of mCPP (Milekic et al., 2006; Valjent et al., 2006), and expression of the transcription factor CREB increases in multiple brain regions after the acquisition of mCPP (Zhou and Zhu, 2006). There is still much to be learned about the mechanisms involved in the formation and maintenance of the associations underlying mCPP, and experiments investigating the mCPP task are the focus of Chapter Two of this dissertation.

In the PCA task, a light or a tone presentation (the CS) is associated with the delivery of sucrose reward (the US) into a reward port, and the rat approaches the port to consume the reward. After learning the association between the CS and US, the rat will approach the reward port during the presentation of the CS before the sucrose reward has been delivered. This task is closely related to a couple of other behavioral paradigms, autoshaping and conditioned orienting, that involve associations between a light or tone presentation and the delivery of a reward, usually a food reward. However, autoshaping and conditioned orienting use different behavioral measures of conditioning than the PCA task, and there is evidence that they involve overlapping but distinct neural circuits (Everitt et al., 1999; Setlow et al., 2002).

There has been little study of the neural circuitry or molecular mechanisms underlying Pavlovian conditioned approach, and much of the data is either negative or conflicting. However, there is some evidence that the amygdala and the NAcc are involved in this task. Intra-amygdala administration of agents that enhance dopamine signaling enhance the acquisition of PCA (Hitchcott et al., 1997a; Hitchcott et al., 1997b). However, lesions of the central nucleus of the amygdala (CeA) and lesions that disconnect the CeA from either substantia nigra pars compacta or the ventral tegmental area do not affect PCA behavior (Gallagher et al., 1990; El-Amamy and Holland, 2007). In addition, lesions disconnecting the basolateral complex of the amygdala (BLA) and the nucleus accumbens (NAcc) impair only second order, but not first order, PCA (Setlow et al., 2002) Excitotoxic lesions of the NAcc core impaired PCA performance, but electrolytic lesions of the core had no effect on behavior (Parkinson et al., 1999; Cassaday et al., 2005). In the NAcc shell, infusions of *d*-amphetamine enhanced the

acquisition of PCA (Phillips et al., 2003), but neither excitotoxic nor electrolytic lesions affected this behavior (Parkinson et al., 1999; Cassaday et al., 2005). Clearly, the circuitry and molecular mechanisms underlying the formation and maintenance of the PCA association are not well understood. Experiments investigating the PCA task are the focus of Chapters Three and Four of this dissertation, and Chapter Four will investigate the role of the NAcc in this task.

Chapter Four also compares the role of the NAcc in the PCA task to its role in a form of aversive Pavlovian conditioning, cued fear conditioning. In the cued fear conditioning task, a discrete stimulus presentation (the CS) is associated with the delivery of a footshock (an aversive US) (Rescorla and Wagner, 1972). The delivery of the footshock causes rats to exhibit a specific defensive reaction known as freezing, where rats crouch in a defensive posture and cease all voluntary movement (LeDoux et al., 1988; LeDoux, 2000, 2003). After learning the association between the CS and the US, rats will exhibit freezing behavior in response to presentation of the CS alone (Rescorla and Wagner, 1972).

Arguably, there is more known about the neurobiology of cued fear conditioning than any other type of Pavlovian conditioning. Multiple experiments using lesions, behavioral pharmacology, molecular biology, and electrophysiological techniques have implicated the amygdala in the learning and expression of cued fear conditioning (LeDoux, 2000, 2003; Kim and Jung, 2006), but there has been disagreement as to whether the amygdala is the site of plasticity underlying the aversive Pavlovian association or is necessary only to modulate plasticity occurring in other brain regions (Fanselow and LeDoux, 1999; McGaugh, 2002). However, the most prominent view of

the neural circuitry underlying cued fear conditioning holds that information about the CS is relayed from sensory areas (such as the auditory thalamus) to the BLA; here it is thought to become associated with information about the US that is also relayed to the BLA (Fanselow and LeDoux, 1999; Kim and Jung, 2006). Output from the BLA is hypothesized to flow to the central nucleus of the amygdala (CeA) and then to the periacqueductal gray (PAG), a brainstem nucleus required for the freezing response (LeDoux et al., 1988; LeDoux, 2000; Kim and Jung, 2006). Although this model has been adjusted slightly in recent years in order to incorporate new behavioral and neuroanatomical data, the fundamentals of this model have remained the same (Paré et al., 2004; Wilensky et al., 2006), and there has been limited investigation into the role that brain regions outside this amygdala-based fear circuit play in the learning and expression of this form of aversive Pavlovian conditioning.

This dissertation will investigate the mechanisms underlying several phases of the formation and maintenance of the Pavlovian conditioning tasks discussed above and will focus mainly on the mechanisms underlying the consolidation and reconsolidation phases of these tasks.

Consolidation and Reconsolidation

Consolidation refers to the process immediately after the initial acquisition of a memory during which the long-term memory is stabilized (Sara, 2000; Dudai, 2004a). The modern concept of consolidation has its origin in the work of Theodore Ribot, a 19th century French physician. Ribot's study of amnesiac patients led him to postulate that retrograde amnesia is governed by a "law of regression" whereby recent memories are

more likely to be lost than older memories (Ribot, 1882). About twenty years later, Müller and Pilzecker noted that human memories require time after acquisition in order to stabilize, and they used the term consolidation to describe this stabilization process (Glickman, 1961; Dudai, 2004a).

Studies of the mechanisms underlying memory consolidation in animals gained popularity in the middle of the twentieth century (Glickman, 1961; McGaugh and Petrinovich, 1965). In most experiments since that time, a specific agent has been said to modulate memory consolidation if administration of the agent immediately after training has an effect on memory but delayed post-training administration has no effect. Since the memory should only need a limited amount of time to consolidate before it is stabilized, agents that affect consolidation should only be able to modulate memory when administered during a discrete time window (Dudai, 2004a).

While the mechanisms underlying the consolidation process can be different for different types of memories, there are some cellular and molecular mechanisms that have been shown to be involved in the consolidation of multiple types of memories in multiple different species. Activation of N-methyl-D-aspartic acid (NMDA) receptors are generally thought to be necessary at the beginning of consolidation (Abel and Lattal, 2001; Tronel and Sara, 2003; Suzuki et al., 2004; Dalley et al., 2005; Ferretti et al., 2005), and activation of dopamine and adrenergic receptors are thought to modulate consolidation (Packard and White, 1989; Setlow and McGaugh, 1998; Miranda et al., 2003; LaLumiere et al., 2004). In fact, amphetamine, which is known to increase the level of catecholamines available in the synapse, has been shown to enhance the consolidation of spatial memory (Packard and White, 1989; Brown et al., 2000), visual

discrimination learning (Krivanek and McGaugh, 1969), aversive conditioning (Doty and Doty, 1966; Haycock et al., 1977; Janak and Martinez, 1992), and verbal learning in humans (Soetens et al., 1993; Soetens et al., 1995).

After activation of receptors, the next step in the consolidation of many memories is thought to be activation of the cAMP-dependent protein kinase (PKA) and the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways (Bourtchouladze et al., 1998; Schafe et al., 1999; Schafe and LeDoux, 2000; Jentsch et al., 2002; Kelley et al., 2003; Purcell et al., 2003; Hawkins et al., 2006). This is thought to lead to subsequent activation of nuclear transcription factors, such as the cAMP response element-binding protein (CREB), that initiate the transcription and translation of multiple effector proteins which alter neuronal growth or structure in a way that stabilizes the long-term memory (Stork and Welzl, 1999; Josselyn et al., 2001; Kida et al., 2002; Hawkins et al., 2006).

Although there is only limited knowledge about the types of effector proteins that are translated and their role in the stabilization and persistence of long-term memories, the general requirement for new protein synthesis during memory consolidation has been studied for decades (Davis and Squire, 1984). In 1963, it was first shown that intracerebral administration of puromycin, an antibiotic that inhibits protein synthesis, prevented learning in an aversive learning task (Flexner et al., 1963). Anisomycin, another protein synthesis inhibitor that prevents peptide bond formation during protein translation (Grollman and Huang, 1973), has been shown to impair the consolidation of a variety of memories (Squire and Davis, 1975; Meiri and Rosenblum, 1998; Schafe et al., 1999; Schafe and LeDoux, 2000; Hernandez et al., 2002). Because anisomycin and other

protein synthesis inhibitors have several side effects and can be toxic when administered systemically, it is important to note that a plethora of control experiments have been conducted over the years to ensure that the memory deficits seen after administration of these drugs is truly due to the inhibition of new protein synthesis and not one of the side effects of the drugs (Davis and Squire, 1984). For example, a structural analogue of anisomycin that has a similar side effect profile but a limited ability to inhibit protein synthesis did not affect memory consolidation (Squire and Barondes, 1974).

When the existence of the consolidation process was first hypothesized, it was postulated that after a memory has been fully consolidated (or stabilized), it should be consistently stable and resistant to amnesic agents such as protein synthesis inhibitors (Dudai, 2004a). However, it has since been shown that some memories exhibit a second period of instability after reactivation of the memory, and it is hypothesized that these memories must undergo a second active process of stabilization, termed reconsolidation, after memory reactivation in order to ensure further maintenance of the memory (Sara, 2000; Dudai, 2004a; Tronson and Taylor, 2007). Although an experiment in 1968 first presented evidence that memories can exhibit a period of lability after memory reactivation (Misanin et al., 1968), investigation of this phenomenon quickly fell out of favor. Interest in the topic of reconsolidation was revived in 2000, however, when it was shown that inhibition of protein synthesis after memory reactivation impaired memory in an auditory fear conditioning task (Nader et al., 2000).

Since the study of reconsolidation is still relatively in its infancy, little about the reconsolidation process is currently understood. Unlike consolidation, however, it is clear that the reconsolidation process does not occur in all memories (Dudai, 2004b).

Factors such as the type of memory, age of memory, and strength of original training can all affect whether or not a memory will exhibit post-reactivation lability (Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Hernandez and Kelley, 2004; Suzuki et al., 2004). Currently, there is no clear understanding of the cellular and molecular mechanisms underlying reconsolidation, but multiple receptors, signaling cascades, and transcription factors have been implicated in the reconsolidation of different types of memories (Tronson and Taylor, 2007). Some of these signaling molecules have been shown to be involved in the reconsolidation of multiple types of memories. For example, blockade of the β-adrenergic receptor has been shown to impair the reconsolidation of cued fear conditioning, spatial learning, and instrumental learning (Przybyslawski et al., 1999; Debiec and LeDoux, 2004; Diergaarde et al., 2006), and the PKA pathway has been shown to be involved in the reconsolidation of both cued fear conditioning and conditioned taste aversion (Koh and Bernstein, 2003; Tronson et al., 2006). Like consolidation, new protein synthesis appears to be generally necessary for the reconsolidation of memories. To date, protein synthesis has been shown to be required for the reconsolidation of a variety of memories, including cued and contextual fear conditioning, inhibitory avoidance, operant conditioning, and conditioned taste aversion, in species ranging from snails to humans (Judge and Quartermain, 1982; Nader et al., 2000; Anokhin et al., 2002; Milekic and Alberini, 2002; Eisenberg et al., 2003; Sangha et al., 2003; Suzuki et al., 2004).

In general, the experiments in Chapters Two and Three of this dissertation will investigate the mechanisms of the consolidation and reconsolidation of appetitive

Pavlovian conditioning. In addition to examining the formation of the Pavlovian associations, Chapter Four will also focus on the expression of Pavlovian conditioning.

References

Abel T, Lattal KM (2001) Molecular mechanisms of memory acquisition, consolidation, and retrieval. Current Opinion in Neurobiology 11:180-187.

Agras WS (2001) The consequences and costs of the eating disorders. Psychiatric Clinics of North America 24:371-379.

Anokhin KV, Tiunova AA, Rose SPR (2002) Reminder effects -- reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. European Journal of Neuroscience 15:1759-1765.

Anthony JC, Warner LA, Kessler RC (1994) Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the national comorbidity survey. Experimental and clinical psychopharmacology 2:244-268.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153:31-43.

Bossert JM, Gray SM, Lu L, Shaham Y (2006) Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. Neuropsychopharmacology 31:2197-2209.

Bourtchouladze R, Abel T, Berman N, Gordon R, Lapidus K, Kandel ER (1998) Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learning and Memory 5:365-374.

Brown RW, Bardo MT, Mace DD, Phillips SB, Kraemer P (2000) D-amphetamine facilitation of Morris water task performance is blocked by eticlopride and correlated with increase dopamine synthesis in the prefrontal cortex. Behavioural Brain Research 114:135-143.

Burattini C, Gill TM, Aicardi G, Janak PH (2006) The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. Neuroscience 139:877-887.

Cassaday HJ, Horsley RR, Norman C (2005) Electrolytic lesions to nucleus accumbens core and shell have dissociable effects on conditioning to discrete and contextual cues in aversive and appetitive procedures respectively. Behavioural Brain Research 160:222-235.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. American Journal of Psychiatry 156:111-118.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci USA 102:6189-6194.

Davis HP, Squire LR (1984) Protein Synthesis and Memory: A Review. Psychological Bulletin 96:518-559.

Debiec J, LeDoux JE (2004) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. Neuroscience 129:267-272.

Diergaarde L, Schoffelmeer ANM, De Vries TJ (2006) B-adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation. Behavioural Brain Research 170:333-336.

Doty BA, Doty LA (1966) Facilitative effects of amphetamine on avoidance conditioning in relation to age and problem difficulty. Psychopharmacologia 9:234-241.

Dudai Y (2004a) The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol 55:51-86.

Dudai Y (2004b) Rites of passage of the engram: reconsolidation and the lingering consolidation hypothesis. Neuron 44:93-100.

Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominance. Science 301:1102-1104.

El-Amamy H, Holland PC (2007) Dissociable effects of disconnecting amygdala central nucleus form the ventral tegmental area or substantia nigra on learned orienting and incentive motivation. European Journal of Neuroscience 25:1557-1567.

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward: the role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 877:412-438.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Ferretti V, Florian C, Costantini VJA, Roullet P, Rinaldi A, De Leonibus E, Oliverio A, Mele A (2005) Co-activation of glutamate and dopamine receptors within the nucleus accumbens is required for spatial memory consolidation in mice. Psychopharmacology 179:108-116.

Flexner JB, Flexner LB, Stellar E (1963) Memory in mice as affected by puromycin. Science 141:57-59.

Gallagher M, Graham PW, Holland PC (1990) The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. J Neurosci 10:1906-1911.

Garavan H, Pankiewicz J, Bloom A, Cho J-K, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. American Journal of Psychiatry 157:1789-1798.

Glickman SE (1961) Perseverative neural processes and consolidation of the memory trace. Psychological Bulletin 58:218-233.

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 22:473-479.

Grollman AP, Huang MT (1973) Inhibitors of protein synthesis in eukaryotes: tools in cell research. Federation Proceedings 32:1673-1678.

Hawkins RD, Kandel ER, Bailey CH (2006) Molecular mechanisms of memory storage in *Aplysia*. Biological Bulletin 210:174-191.

Haycock JW, Van Buskirk R, Gold PE (1977) Effects on retention of posttraining amphetamine injections in mice: interaction with pretraining experience. Psychopharmacology 54:21-24.

Heather N, Stallard A, Tebbutt J (1991) Importance of substance cues in relapse among heroin users: comparison of two methods of investigation. Addictive behaviors 16:41-49.

Hernandez PJ, Kelley AE (2004) Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. Learning and Memory 11:748-754.

Hernandez PJ, Sadeghian K, Kelley AE (2002) Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. Nature Neuroscience 5:1327-1331.

Hitchcott PK, Bonardi CMT, Phillips GD (1997a) Enhanced stimulus-reward learning by intra-amygdala administration of a D_3 dopamine receptor agonist. Psychopharmacology 133:240-248.

Hitchcott PK, Harmer CJ, Phillips GD (1997b) Enhanced acquisition of discriminative approach following intra-amygdala d-amphetamine. Psychopharmacology 132:237-246.

Janak PH, Martinez JL, Jr. (1992) Cocaine and amphetamine facilitate retention of jump-up responding in rats. Pharmacology, Biochemistry and Behavior 41:837-840.

Jansen A (1998) A learning model of binge eating: cue reactivity and cue exposure. Behaviour Research and Therapy 36:257-272.

Jentsch JD, Olausson P, Nestler EJ, Taylor JR (2002) Stimulation of protein kinase A activity in the rat amygdala enhances reward-related learning. Biological Psychiatry 52:111-118.

Josselyn SA, Shi C, Carlezon WA, Jr., Neve RL, Nestler EJ, Davis M (2001) Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. Journal of Neuroscience 21:2404-2412.

Judge ME, Quartermain D (1982) Characteristics of retrograde amnesia following reactivation of memory in mice. Physiology and Behavior 28:585-590.

Kelley Á, Laroche S, Davis S (2003) Activation of mitogen-activated protein kinase/extracellular signal-related kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. Journal of Neuroscience 12:5354-5360.

Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, Silva AJ (2002) CREB required for the stability of new and reactivated fear memories. Nature Neuroscience 5:348-355.

Kim JJ, Jung MW (2006) Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neuroscience and Biobehavioral Reviews 30:188-202.

Koh MT, Bernstein IL (2003) Inhibition of protein kinase A activity during conditioned taste aversion retrieval: interference with extinction or reconsolidation of a memory? Neuroreport 14:405-407.

Krivanek JA, McGaugh JL (1969) Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. Agents and Actions 1:36-42.

LaLumiere RT, Nguyen PV, McGaugh JL (2004) Post-training intrabasolateral amygdala infusions of dopamine modulate consolidation of inhibitory avoidance memory: involvement of noradrenergic and cholinergic systems. European Journal of Neuroscience 20:2804-2810.

Ledgerwood DM, Petry NM (2006) What do we know about relapse in pathological gambling? Clinical Psychology Review 26:216-228.

LeDoux JE (2000) Emotion circuits in the brain. Annual Reviews in Neuroscience 23:155-184.

LeDoux JE (2003) The emotional brain, fear, and the amygdala. Cellular and Molecular Neurobiology 23:727-738.

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. Journal of Neuroscience 8:2517.

McGaugh JL (2002) Memory consolidation and the amygdala: a systems perspective. Trends in Neurosciences 25:456-461.

McGaugh JL, Petrinovich LF (1965) Effects of drugs on learning and memory. International Review of Neurobiology 8:139-196.

McKay JR, Weiss RV (2001) A review of the temporal effects and outcome predictors in substance abuse treatment studies with long-term follow-ups. Evaluation Review 25:113-161.

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behavioural Brain Research 87:139-148.

Meiri N, Rosenblum K (1998) Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. Brain Research 789:48-55.

Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. Neuron 36:521-525.

Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. J Neurosci 26:3010-3020.

Miranda MI, LaLumiere RT, Buen TV, Bermudez-Rattoni F, McGaugh JL (2003) Blockade of noradrenergic receptors in the basolateral amygdala impairs taste memory. European Journal of Neuroscience 18:2605-2610.

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160:554-555.

Nader K, Schafe GE, LeDoux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406:722-726.

Nie H, Janak PH (2003) Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology 168:222-228.

Office of National Drug Control Policy (2007) Drug Control Strategy: Fiscal Year 2008 Budget Summary. Washington, DC: Office of the President.

Olmstead MC, Franklin KBJ (1997) The Development of a conditioned place preference to morphine: effects of lesions of various CNS sites. Behavioral Neuroscience 111:1313-1323.

Packard MG, White NM (1989) Memory facilitation produced by dopamine agonists" role of receptor subtype and mnemonic requirements. Pharmacology, Biochemistry and Behavior 33:511-518.

Paré D, Quirk GJ, LeDoux JE (2004) New vistas on amygdala networks in conditioned fear. Journal of Neurophysiology 92:1-9.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by d-amphetamine. J Neurosci 19:2401-2411.

Pavlov IP (1927) Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. London: Oxford University Press.

Pedreira ME, Maldonado H (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. Neuron 38:863-869.

Phillips GD, Setzu E, Hitchcott PK (2003) Facilitation of Appetitive Pavlovian Conditioning by *d*-Amphetamine in the Shell, but Not the Core, of the Nucleus Accumbens. Behavioral Neuroscience 117:675-684.

Piasecki TM (2006) Relapse to smoking. Clinical Psychology Review 26:196-215.

Przybyslawski J, Roullet P, Sara SJ (1999) Attenuation of emotional and nonemotional memories after their reactivation: role of β adrenergic receptors. Journal of Neuroscience 19:6623-6628.

Purcell AL, Sharma SK, Bagnall MW, Sutton MA, Carew TJ (2003) Activation of a tyrosine kinase-MAPK cascade enhances the induction of long-term synaptic facilitation and long-term memory in *Aplysia*. Neuron 37:473-484.

Rescorla RA, Wagner AR (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and non-reinforcement. . In: Classical conditioning II: Current research and theory (Black AH, Proskasy WF, eds), pp 64-69. NY: Appleton-Century-Crofts.

Ribot T (1882) Diseases of Memory. In: Diseases of Memory, Diseases of the Will, and Diseases of Personality, pp 1-48. New York: Humboldt Publishing Co.

Rohsenow DJ, Monti PM, Rubonis AV, Sirota AD, Niaura RS, Colby SM, Wunschel SM, Abrams DB (1994) Cue reactivity as a predictor of drinking among male alcoholics. Journal of Consulting and Clinical Psychology 62:620-626.

Rossi NA, Reid LD (1976) Affective states associated with morphine injections. Physiological Psychology 4:269-274.

Sangha S, Scheibenstock A, Lukowiak K (2003) Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of right pedal dorsal 1. Journal of Neuroscience 23:8034-8040.

Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. Learning and Memory 7:73-84.

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. Journal of Neuroscience 20:1-5.

Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learning and Memory 6:97-110.

See RE (2002) Neural substrates of conditioned-cued relapse to drug-seeking behavior. Pharmacology, Biochemistry and Behavior 71:517-529.

See RE, Kruzich PJ, Grimm JW (2001) Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. Psychopharmacology 154:301-310.

Setlow B, McGaugh JL (1998) Sulpiride infused into the nucleus accumbens posttraining impairs memory of spatial water maze training. Behavioral Neuroscience 112:603-610.

Setlow B, Holland PC, Gallagher M (2002) Dissconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive Pavlovian second-order conditioned response. Behavioral Neuroscience 116:267-275.

Sobik L, Hutchison K, Craighead L (2005) Cue-elicited craving for food: a fresh approach to the study of binge eating. Appetite 44:253-261.

Soetens E, D'Hooge R, Hueting JE (1993) Amphetamine enhances human-memory consolidation. Neuroscience Letters 161:9-12.

Soetens E, Casaer S, D'Hooge R, Hueting JE (1995) Effect of amphetamine on long-term retention of verbal material. Psychopharmacology 119:115-162.

Squire LR, Barondes SH (1974) Anisomycin, like other inhibitors of cerebral protein synthesis, impairs 'long-term' memory of a discrimination task. Brain Research 66:301-308.

Squire LR, Davis HP (1975) Cerebral protein synthesis inhibition and discrimination training: effects of extent and duration of inhibition. Behavioral Biology 13:49-57.

Stapleton J (1998) Cigarette smoking prevalence, cessation and relapse. Statistical Methods in Medical Research 7:187-203.

Stork O, Welzl H (1999) Memory formation and the regulation of gene expression. Cellular and Molecular Life Sciences 55:575-592.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787-4795.

Tronel S, Sara SJ (2003) Blockade of NMDA receptors in prelimbic cortex induces an enduring amnesia for odor-reward associative learning. Journal of Neuroscience 23:5472-5476.

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nature Reviews Neuroscience 8:1-14.

Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nature Neuroscience 9:167-169.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress, and new issues. Progress in Neurobiology 56:613-672.

United Nations Office on Drugs and Crime (2003) Global Illicit Drug Trends. Vienna, Austria: United Nations Office on Drugs and Crime.

Valjent E, Corbille A-G, Bertran-Gonzalez J, Herve D, Girault J-A (2006) Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. PNAS 103:2932-2937.

Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE (2006) Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. Journal of Neuroscience 26:12387-12396.

Witkiewitz K, Marlatt GA (2004) Relapse prevention for alcohol and drug problems: that was zen, this is tao. American Psychologist 59:224-235.

Zhou L-F, Zhu Y-P (2006) Changes of CREB in rat hippocampus, prefrontal cortex and nucleus accumbens during three phases of morphine induced conditioned place preference in rats. Journal of Zhejiang University Science B 7:107-113.

Zironi I, Burattini C, Aicardi G, Janak PH (2006) Context is a trigger for relapse to alcohol. Behavioural Brain Research 167:150-155.

Chapter Two:

Post-training and Post-reactivation Administration of Amphetamine Enhances Morphine Conditioned Place Preference

Abstract

Amphetamine has been shown to enhance consolidation in a variety of memory paradigms. However, it is not known if amphetamine can modulate the consolidation of the types of context-reward associations involved in drug addiction, such as those formed in the conditioned place preference (CPP) task. Also, some types of memory exhibit a second period of lability following memory reactivation, and it is not known whether amphetamine administered during this period can modulate CPP. Our study investigated whether amphetamine can enhance morphine CPP when administered during the consolidation period or the post-reactivation period. Subjects were trained in the CPP task and injected with amphetamine or vehicle immediately or six hours after each training session. The day after the completion of training, they were tested. Amphetamine injected immediately but not six hours after training enhanced morphine CPP. In separate experiments, subjects were first trained in the CPP task. The day following the completion of training, subjects were given a memory reactivation session and injected with amphetamine or vehicle immediately or six hours after reactivation.

Subjects were tested the next day. Amphetamine injected immediately but not six hours after memory reactivation enhanced morphine CPP. However, amphetamine injected without memory reactivation had no effect on the expression of morphine CPP. Our results suggest that amphetamine enhances the consolidation of morphine CPP and that morphine CPP exhibits a temporally limited period of post-reactivation lability during which the memory can be modulated.

Introduction

The consolidation period of a memory refers to a temporally limited period of lability immediately following acquisition during which a long-term memory is thought to be forming. Many pharmacologic agents have been shown to modulate consolidation in a variety of memory tasks (for reviews, see Kesner and Ellis, 1983; McGaugh, 2000; Kelly et al. 2003). These agents modulate memory when administered immediately after training, but they have no effect when administered several hours later. Amphetamine (AMPH) is one agent that has been shown to enhance the consolidation of a variety of memory types, including spatial memory, active avoidance conditioning, and verbal declarative memory (Krivanek and McGaugh, 1969; Janak an Martinez, 1992; Soetens et al., 1995; Oscos et al., 1998). However, it is not known if AMPH can also modulate the consolidation of the learned associations between a context and reward that are formed in the conditioned place preference (CPP) task.

The CPP task tests an animal's ability to associate a drug reward with a unique environmental context. In this task, subjects are repeatedly exposed to pairings of a distinct environment with the administration of a drug. During a drug-free test, subjects

are said to exhibit a conditioned place preference if they spend more time in the drug-associated environment than in an environment that has not been associated with the drug.

Although the CPP task has most commonly been used to determine the rewarding properties of drugs of abuse and natural stimuli (for review, see Tzschentke, 1998), this task may also be used to investigate the mechanisms underlying context-reward associations (Hsu et al., 2002). Because there is no drug onboard during the test sessions, the expression of CPP must rely on an association between the drug reward and the environmental context of the drug-associated chamber.

Understanding the neural mechanisms of context-reward associations is important because of the influence that drug-associated environmental contexts and cues can exert on drug-seeking behavior. Both environmental contexts and specific environmental cues associated with a drug can consistently reinstate drug-seeking in abstinent rats (for reviews, see See, 2002; Shalev et al., 2002; Shaham et al, 2003). In humans, drug-associated stimuli increase self-reported levels of drug craving in addicts (Maas et al., 1988; Grant et al., 1996; Childress et al, 1999; Garavan et al., 2000) and can be an important factor in precipitating relapse in addicts (Heather et al, 1991; Shiffman et al., 2002).

Few studies have investigated the mechanisms underlying the consolidation of CPP. Protein kinases A and C are involved in the consolidation of cocaine CPP (Cervo et al, 1997), and the amygdala, including muscarinic receptors within the amygdala, may play a role in the consolidation of amphetamine CPP (Hsu et al., 2002; Schroeder and Packard, 2002). However, there have been no studies investigating whether the

consolidation of CPP can be positively modulated. Therefore, the first part of our study investigates whether amphetamine can enhance the consolidation of morphine CPP.

In addition to the consolidation period, some types of memory also exhibit a temporally limited period of lability immediately following reactivation of the memory (for review, see Sara, 2000). This post-reactivation lability has been observed in a variety of memory types across several species including rodents (Misanin et al., 1968; Judge and Quartermain, 1982; Przybyslawski and Sara, 1997; Przybyslawski et al., 1999; Nader et al., 2000; Kida et al., 2002; Milekic and Alberini, 2002; Bozon et al, 2003; Kelly et al., 2003; Koh and Bernstein, 2003), chicks (Anokhin et al., 2002), fish (Eisenberg et al., 2003), crabs (Pedreira and Maldonado, 2003), and humans (Kleim et al., 2003; Walker et al, 2003), and it has recently been observed in two different types of addiction-related learning (Lee et al., 2005; Miller and Marshall, 2005). Lee et al. (2005) showed that zif268, an immediate early gene, is required in the basolateral amygdala after rexposure to a cocaine-associated conditioned stimulus in order for the stimulus to maintain its reinforcing properties. In addition, Miller and Marshall (2005) recently showed that post-reactivation inhibition of MAP/ERK kinase (MEK) in the nucleus accumbens core disrupts the memory for a cocaine CPP. However, it is not known whether morphine CPP exhibits this type of post-reactivation lability, and there has been little investigation of whether any memory can be positively modulated (rather than impaired) during the post-reactivation period. To address this question, the second part of our study investigates whether amphetamine can enhance CPP when administered immediately after reactivation of the association.

Materials and Methods

Animals

All studies used male rats (weighing between 250-280 g at the beginning of the experiments) that received food and water ad libitum and were housed individually in polycarbonate cages in ventilation racks (Biozone, Fort Mill, SC, USA). Subjects were kept on a 12 hour light/dark cycle with lights on at 6 a.m., and all behavioral procedures were conducted during the light cycle. These procedures were approved by the Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center at the University of California, San Francisco, and are in accordance with "PHS Policy on Humane Care and Use of Laboratory Animals", Office of Laboratory Animal Welfare, National Institutes of Health, USA, revised 2002.

Apparatus

Each place preference apparatus (Med Associates, VA) contained three chambers separated by sliding doors. The two main chambers (28 x 21 x 21 cm) differed in color (black vs. white), lighting (white vs. red), and floor texture (metal bars vs. metal grid) and were connected by a middle chamber (12 x 21 x 21 cm) that was grey in color. The time spent in each chamber was automatically recorded by infrared beam breaks.

Drugs

Morphine (Sigma; St. Louis, MO) was dissolved in physiological sterile saline and injected subcutaneously (SC) at a dose of 10 mg/kg. This dose was chosen based on

previous studies of morphine place conditioning (Bardo et al., 1995). d-Amphetamine (Sigma; St. Louis, MO) was dissolved in physiological sterile saline and injected into the intraperitoneal cavity (IP) at a dose of 1 mg/kg. This dose of amphetamine was chosen based on its ability to enhance memory consolidation in another behavioral paradigm (Janak and Martinez, 1992).

Behavioral procedures

Experiment 1: What is the effect of AMPH on memory for morphine CPP?

Experiment 1a: Post-training injections of amphetamine

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were first given a baseline session in which they were placed in the middle chamber for a 60 second adaptation period. The doors to the other chambers were then raised, and subjects were allowed to explore all three chambers for thirty minutes. The time spent in each chamber was recorded.

Training sessions were then conducted once a day for six consecutive days. On alternating days, rats were given injections of morphine or saline immediately before being placed in the middle chamber for a 30 second adaptation period and then confined for thirty minutes to the morphine-paired or saline-paired chamber, respectively. Rats received an injection of either amphetamine or vehicle immediately after each training session. Subjects were sorted into experimental groups semi-randomly: groups were counterbalanced for drug-paired chamber, days receiving drug vs. saline, CPP box, and the order in which rats experienced behavioral sessions.

A test session identical to the baseline session was then conducted 24 hours after the last training session. A subset of subjects experienced four more test sessions, each separated by 24 hours.

Experiment 1b: Post-reactivation injections of amphetamine

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were conditioned as described for Experiment 1a, except that 8 training sessions (4 morphine, 4 saline) were given. We increased the number of training sessions for this experiment in order to increase the probability that animals would develop a more robust memory which would be less likely to extinguish during the subsequent reactivation session. Twenty-four hours after the last training session, subjects were given their first 30 minute test, in which time spent in each side of the apparatus was measured. This test served as a reactivation of the memory for CPP. Subjects then received an injection of amphetamine or vehicle immediately after the memory reactivation. Rats were divided into experimental groups in the manner described for the first experiment.

Subjects were given a 30 minute test session 24 hours after the memory reactivation. A subset of subjects also experienced two subsequent test sessions separated by 24 hours.

Experiment 2: Is the effect of AMPH temporally limited?

Experiment 2a: Post-training injections of AMPH

Male CD rats (Charles River, Wilmington, MA, USA) were conditioned as described for Experiment 1a, except that 4 training sessions (2 morphine, 2 saline) were given. Because we found that animals from Charles River required fewer training sessions to

develop a level of conditioning similar to the animals from Harlan (Blaiss and Janak, unpublished data), subjects in Experiment 2 were given fewer training sessions than in the analogous parts of Experiment 1. After each training session, all subjects received 2 injections. One group of animals received an injection of amphetamine immediately after each session and a vehicle injection 6 hrs after each session; the drugs were reversed for a second group of animals. Subjects were given a 30 minute test session 72 hours after the last training session. Rats were divided into experimental groups in the manner described for the first experiment, and any rat that had a difference score (see data analysis section) greater than 250 seconds during the baseline session was dropped from the study.

Experiment 2b: Post-reactivation injections of AMPH

Male CD rats (Charles River, Wilmington, MA, USA) were conditioned as described for Experiment 1a with 6 training sessions, and twenty-four hours after the last training session, subjects were given a memory reactivation test as described in Experiment 1b. After the memory reactivation, all rats received 2 injections. One group received an injection of amphetamine immediately after the memory reactivation and a vehicle injection 6 hrs after the memory reactivation; in a second group the drugs were reversed. Subjects were then given a 30 minute test session 24 hours after the memory reactivation.

Rats were divided into experimental groups in the manner described for the first experiment, and any rat that did not exhibit a CPP (difference score greater than zero) during the memory reactivation was also dropped from the study.

Experiment 3: Control – Is memory reactivation necessary for CPP enhancement?

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were conditioned as described for Experiment 1b. Twenty-four hours after the last training session, subjects were transported to the behavioral testing room, given an injection of amphetamine or vehicle, and then returned to their home cages. On the next day, subjects were given one 30 minute test session. Rats were divided into experimental groups in the manner described for the first experiment.

Experiment 4: Control – Does AMPH also enhance the consolidation of morphine CPP when morphine is injected after (instead of before) training sessions?

Male CD rats (Charles River, Wilmington, MA, USA) were initially given a baseline session. Training sessions were then conducted once a day for 4 consecutive days. On alternating days, rats were given injections of morphine or saline immediately *after* being confined for thirty minutes to the morphine-paired or saline-paired chamber, respectively. Subjects were also injected with either vehicle or amphetamine immediately after every training session. A test session identical to the baseline session was then conducted 72 hours after the last training session. Rats were divided into experimental groups in the manner described for the first experiment, and any rat that had a difference score (see data analysis section) greater than 250 seconds during the baseline session was dropped from the study.

Data Analysis

During test sessions and the memory reactivation session, the time the subjects spent in each chamber was recorded by computer, and the difference in time spent in the drug-paired chamber and saline-paired chamber (difference score) was taken as a measure of place conditioning. To determine whether subjects exhibited an inherent preference during the baseline session, the total time spent in the chamber to be paired with morphine was compared to the total time spent in the chamber to be paired with saline using paired t-tests. Data was analyzed in the same way (the time spent in the morphine-paired chamber compared to the time spent in the saline-paired chamber) to determine whether trained subjects exhibited a significant conditioned place preference. In order to determine whether there was an effect of Treatment during a single test session, we conducted 1-way ANOVAs with Treatment as a between-subjects factor. Data from subjects who received multiple test sessions were analyzed using 2-way repeated measures ANOVAs; ANOVAs were conducted with Treatment as a between-subjects factor and Test Session as a within-subjects factor.

Results

Experiment 1: Amphetamine enhances memory for morphine CPP

Experiment 1a: Post-training injections of amphetamine enhance morphine CPP

Rats were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 691.65 ± 23.69 seconds; mean (±SEM) time in the chamber to be paired

with saline: 645.48 ± 26.96 seconds; paired *t*-test, t(27) = 1.13, P=.27]. Then, to determine whether amphetamine can enhance the consolidation of morphine CPP, subjects were injected with either AMPH or vehicle immediately after every CPP training session. This included sessions in which rats were injected with morphine and confined to the morphine-associated chamber as well as sessions in which rats were injected with saline and confined to the saline-associated chamber. Subjects were then allowed to explore all chambers during a test session, and rats that received injections of AMPH immediately after training sessions showed an enhanced level of morphine CPP upon testing when compared to subjects that received vehicle. Specifically, the AMPH group had a greater mean difference score when compared to the vehicle group (Figure 1a; one-way ANOVA, F(1,26)=5.94, P<.03). As shown in Figure 1b**,** this effect of AMPH persisted across four subsequent tests for the subset of subjects that was repeatedly tested. While the vehicle group no longer showed a significant CPP by Test 3 [mean (±SEM) time in the morphine chamber: 717.24 ± 55.86 seconds; mean (±SEM) time in the saline chamber: 587.52 ± 86.19 seconds; paired *t*-test, t(5) = 1.13, P=.31], the AMPH group's place preference remained significant even on Test 5 [mean (±SEM) time in the morphine chamber: 860.39 ± 88.03 seconds; mean (±SEM) time in the saline chamber: 458.81 ± 75.02 seconds; paired *t*-test, t(5) = 2.97, P<.031]. A within-subject repeated measures ANOVA found a main effect of Treatment and Test Session across all five tests [Treatment: F(1,10)=7.85, P<.02; Test Session: F(4,10)=2.83, P<.04], but there was no significant interaction between Treatment and Test Session, suggesting that although the overall performance changed over time, the degree of difference between groups was not significantly different across the repeated tests.

Experiment 1b: Post-reactivation injections of amphetamine enhance morphine CPP

Subjects were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 656.99 ± 19.48 seconds; mean (±SEM) time in the chamber to be paired with saline: 676.78 ± 21.97 seconds; paired *t*-test, t(27) = -.51, P=.61]. To test whether post-reactivation administration of AMPH can enhance morphine CPP, rats were given CPP training sessions and a memory reactivation session. Figure 2a shows the level of morphine CPP seen during the memory reactivation, after subjects have been divided into treatment groups, but before amphetamine has been administered. There was no statistical difference between groups (AMPH group vs. vehicle group) during this session [one-way ANOVA; F(1,26)=.67, P=.42]. However, immediately after this memory reactivation session, rats were injected with either vehicle or AMPH, and during a test session 24 hours later, the AMPH group showed enhanced morphine CPP when compared to the vehicle group [Figure 2b; one-way ANOVA; F(1,26)=4.98, P<.04]. In addition, for the subset of subjects that was repeatedly tested, the AMPH group exhibited enhanced CPP over two subsequent tests (Figure 2c); a within-subjects repeated measures ANOVA found a main effect of Treatment [F(1,10)=5.15; P<.05]. Similar to experiment 1a, there was no significant interaction between Treatment and Test Session, indicating that the degree of difference between groups was not significantly different across the repeated tests.

Experiment 2: The memory enhancing effect of amphetamine is temporally limited

Experiment 2a: Immediate, but not delayed, post-training injections of amphetamine enhance morphine CPP

Rats were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 633.09 ± 19.29 seconds; mean (±SEM) time in the chamber to be paired with saline: 619.86 ± 14.72 seconds; paired *t*-test, t(29)=.46, P=.65]. To investigate whether the memory enhancing effect of post-training injections of AMPH is temporally limited, we asked whether AMPH injected 6 hours after training can enhance the consolidation of morphine CPP in a manner similar to immediate post-training injections of AMPH. Because stress can modulate memory (for review, see Wolf, 2003), we designed this study to control for the possibility that injection stress occurring at different times in the different groups could act as a confounding factor. Therefore, all rats were given injections both immediately and 6 hours after each training session. One group received an injection of AMPH immediately after training and a vehicle injection 6 hours after training; in the second group, the conditions were reversed. This serves to ensure that our results stem from an effect of the drug rather than any possible effects of injection stress. During a test session, the group that received AMPH immediately after training showed enhanced CPP when compared to the group that received AMPH 6 hrs after training [Figure 3a; one-way ANOVA; F(1,28)=7.47, P<.02].

Experiment 2b: Immediate, but not delayed, post-reactivation injections of amphetamine enhance morphine CPP

Subjects were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 621.35 ± 17.23 seconds; mean (±SEM) time in the chamber to be paired with saline: 637.50 ± 19.88 seconds; paired *t*-test, t(21) = -.56, P=.59]. To test whether the enhancing effect of post-reactivation injections of AMPH is temporally limited, rats were then given CPP training sessions and a memory reactivation session. Figure 4a shows the level of morphine CPP seen during the memory reactivation, after subjects have been divided into treatment groups, but before amphetamine has been administered. There was no statistical difference between groups (AMPH -- immediate injections vs. AMPH -- delayed injections) during this session [one-way ANOVA; F(1,20)=.22, P=.65]. All rats were given injections both immediately and 6 hours after this memory reactivation session. One group received an injection of AMPH immediately after reactivation and a vehicle injection 6 hours after reactivation; in the second group, the conditions were reversed. As in Experiment 2a, this design ensures that our results stem from an effect of the drug rather than any possible effects of injection stress. During the test session 24 hours later, the group that received AMPH immediately after memory reactivation showed enhanced morphine CPP when compared to the group that received AMPH 6 hrs after memory reactivation [Figure 4b; one-way ANOVA; F(1,20)=6.58, P<.02].

Experiment 3: After the memory is formed, reactivation is necessary for amphetamine to enhance morphine CPP

In this control study, we investigated whether the enhancing effect of AMPH seen in experiments 1b and 2b required reactivation of the memory. Subjects showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 679.56 ± 34.48 seconds; mean (±SEM) time in the chamber to be paired with saline: 649.64 ± 31.17 seconds; paired *t*-test, t(15)=.49, P=.63]. Rats were given CPP training sessions, but unlike the subjects in experiments 1b and 2b, they did not experience a memory reactivation session. Instead of a memory reactivation session, they were simply transported to the behavioral testing room, given an injection of AMPH or vehicle, and returned to their home cages. During the test the next day, there was no statistical difference between the groups' difference scores [mean (±SEM) difference score for AMPH group (n=8): 465.25 ± 90.24 seconds; mean (±SEM) difference score for vehicle group (n=8): 414.10 ± 57.24 seconds; one-way ANOVA; Treatment: F(1,14)=.229, P=.64], suggesting that memory reactivation is necessary for AMPH to enhance morphine CPP.

Experiment 4: Amphetamine does not enhance morphine CPP consolidation when morphine is injected after (instead of before) training sessions.

In Experiments 1a and 2a, AMPH was administered after each training session. If it is possible for rats to develop a morphine CPP without having morphine present during the actual training session, then it also might be possible that our results in Experiments 1a

and 2a were due to a drug-drug interaction between morphine and amphetamine that enhances the rewarding properties of morphine (see Discussion). Therefore, in this control study, we investigated whether amphetamine could enhance the consolidation of morphine CPP when morphine or saline was injected after training sessions (i.e. after confinement to the appropriate chamber) rather than before training sessions. During the baseline session, rats showed no natural preference for either of the conditioning chambers [mean (±SEM) time in the chamber to be paired with morphine: 636.74 ± 35.74 seconds; mean (±SEM) time in the chamber to be paired with saline: 631.00 ± 26.29 seconds; paired *t*-test, t(15)=0.15, P=.88]. During alternating training sessions, all subjects were given injections of morphine or saline immediately *after* being confined to the morphine-paired or saline-paired chamber, respectively. Subjects were injected with either vehicle or AMPH immediately after each training session. Upon testing, there was no difference between the vehicle group and the AMPH group [mean (±SEM) difference score for vehicle group (n=8): 164.23 ± 153.33 seconds; mean (±SEM) difference score for the AMPH group (n=8): 133.71 ± 131.44 seconds; one-way ANOVA; F(1,14)=.02, P=.88]. In fact, neither group exhibited a statistically significant level of CPP during the test session (see Table 1).

Discussion

Our study found that amphetamine injected immediately, but not 6 hrs, after training enhances morphine CPP, indicating that amphetamine enhances the consolidation of morphine CPP. In addition, we have shown that amphetamine injected immediately, but not 6 hrs, after memory reactivation also enhances morphine CPP, suggesting that

morphine CPP exhibits a temporally limited period of post-reactivation lability during which the memory can be modulated. These findings demonstrate that the consolidation of morphine CPP can be modulated in a manner similar to other types of memory, provide evidence that memories can be positively modulated after reactivation, and suggest the possibility of a novel line of inquiry into the treatment of addiction.

Amphetamine enhances the consolidation of morphine CPP

In Experiment 1a, we showed that AMPH injected immediately after training enhances the expression of morphine CPP. In addition, memory for CPP was enhanced not only during the first test but during five tests across five consecutive days. The effect of AMPH is therefore not transitory; there is a persistent effect of the drug. Also, in order to have an effect, AMPH must be injected within a discrete time window after training. In Experiment 2a, we showed that AMPH enhances the expression of morphine CPP only when injected immediately, but not 6 hours, after training. Therefore, the results from Experiments 1a and 2a together suggest that amphetamine enhances the consolidation of the association between morphine and the drug-paired chamber.

An alternative explanation is that amphetamine is not enhancing the memory for morphine CPP but is instead enhancing the hedonic or rewarding properties of morphine. However, two aspects of the study design argue against this possibility. First, amphetamine was administered after every training session, including those sessions where subjects were injected with saline and exposed to the saline-associated chamber. Second, amphetamine was administered after (rather than before) each training session. Therefore, the hedonic effect of morphine could not have been altered during the training session when subjects had the opportunity to experience both the hedonic effect of

morphine and the morphine-associated environment and to associate them. However, this argument rests on the assumption that morphine must be present during the actual training session in order for an animal to develop a morphine CPP. We tested that assumption directly in Experiment 4. In that experiment, we showed that when morphine is injected *after* training sessions (so that subjects never experience the pharmacological effects of morphine until *after* they have been exposed to the drug-paired chamber), amphetamine does not enhance morphine CPP, and in fact, these rats do not even develop a morphine CPP. In addition, Gaiardi, et al. (1998) have shown that concomitant administration of AMPH (at doses of .5, 1, and 2 mg/kg) does not affect the level of morphine CPP (at morphine doses of 5 and 20 mg/kg), suggesting that there is no synergistic interaction between the two drugs that affects CPP. Finally, our results in Experiments 1b and 2b show that amphetamine can enhance morphine CPP when administered after a test session with no drug onboard, indicating that amphetamine can enhance memory for morphine CPP independent of a pharmacological interaction with morphine. Therefore, the combination of the above arguments suggests that it is highly unlikely that our results in Experiments 1a and 2a are due to an enhancement of the rewarding properties of morphine.

Post-reactivation administration of amphetamine enhances morphine CPP

In Experiment 1b, we show that AMPH injected immediately after memory reactivation enhances morphine CPP. This enhancement of morphine CPP also persisted across multiple test sessions, suggesting that the long-term memory for the association was enhanced and that the results cannot be explained by a transitory effect of AMPH on

memory retrieval. In Experiment 2b, we show that the enhancing effect of AMPH is temporally limited; AMPH enhances morphine CPP when injected immediately, but not 6 hours, after memory reactivation. We then conducted a control study (Experiment 3) in which subjects were injected with amphetamine 24 hrs before the CPP test, without memory reactivation. In these subjects, amphetamine had no effect on CPP. This indicates that amphetamine's enhancement of CPP when administered post-reactivation is not a general effect of the drug, but rather requires reactivation of the memory. The combined results of these experiments suggest that after reactivation of the memory for the morphine CPP, there is a temporally limited period of post-reactivation lability during which the memory can be modulated. Although other types of memory have also been shown to exhibit a period of post-reactivation lability (see Introduction), our results are a rare example of positive modulation of a memory during this period.

Another possible interpretation of these experiments is that AMPH is blocking the consolidation of an extinction memory rather than enhancing the memory of the original association. Our memory reactivation session is inherently an extinction session, and by test 1, rats that received a vehicle injection after reactivation no longer showed a significant difference between the time spent in the morphine-paired chamber and the saline-paired chamber (see Table 1). On the other hand, during the same test, rats that received an AMPH injection immediately after reactivation still showed a significant difference between the time spent in the morphine- and saline-paired chambers. Recent studies of fear conditioning and conditioned taste aversion have shown that the same post-reactivation manipulation can either modulate the original learning or new extinction learning, depending on relatively small changes in the behavioral protocol (Eisenberg et

al., 2003; Pedreira and Maldonado, 2003), and it is possible that future studies using different behavioral protocols could determine for certain whether our results are due to a modulation of the original memory or the new extinction memory. However, because AMPH has been shown consistently to enhance a wide variety of memory types across multiple species (see Introduction) but has rarely been shown to block any type of memory in any species, the most parsimonious interpretation of our data is that post-reactivation injections of AMPH enhance the original memory.

It should also be noted that during the memory reactivation (before the injection of amphetamine) in Experiment 1b, the group that was to receive amphetamine immediately after reactivation exhibited a slightly higher mean difference score than the group that was to receive vehicle immediately after reactivation. Although this difference is not statistically significant (see Results), it could still be argued that the difference between groups during the tests is simply an amplification of this initial slight difference. However, there is no correlation between the level of CPP exhibited during the memory reactivation and during the following test ($n=28$; $R=.303$; $P>.1$). This suggests that our results are due to the effect of amphetamine rather than any inherent difference between groups.

Possible Mechanisms

Further studies are needed to determine the mechanisms by which post-training and post-reactivation injections of amphetamine act to enhance morphine CPP. Studies investigating the mechanisms underlying the memory enhancing effects of amphetamine in other behavioral paradigms have implicated both the norepinephrine and dopamine systems (Lee and Ma, 1995; Brown et al., 2000), including multiple types of dopamine

receptors (Fenu and Di Chiara, 2003; Phillips et al., 2002). Noradrenergic and domaminergic signaling have also been shown to modulate consolidation in a variety of learning and memory paradigms ranging from the Morris water maze to the conditioned taste aversion task (Kesner and Ellis, 1983; Setlow and McGaugh, 1998; LaLumiere et al., 2003; Miranda et al., 20033; LaLumiere et al., 2004; Dalley et al., 2005). Although the role of these neurotransmitter systems in reconsolidation has not been as extensively characterized, the D1 receptor antagonist SCH23390 has been shown to impair the reconsolidation of an inhibitory avoidance task in day-old chicks (Sherry et al., 2005), and the β-adrenergic receptor antagonist proponalol has been shown to impair the reconsolidation of auditory fear conditioning, inhibitory avoidance, and a spatial memory task in rats (Pryzybyslawski et al., 1999; Debiec and LeDoux, 2004). It is possible that amphetamine is acting through these neurotransmitter systems to produce our results.

Although AMPH had the same effect during the consolidation and post-reactivation period, the degree of similarity in the mechanisms underlying AMPH's effect during these two phases is currently unclear. In other memory paradigms, the nature of the post-reactivation lability, the mechanisms underlying it, and its degree of similarity to the consolidation period are only beginning to be investigated. Some molecules that are necessary during the consolidation period also appear to be necessary during the post-reactivation period for continued maintenance of the memory (Przybyslawski and Sara, 1997; Przybyslawski et al., 1999; Nader et al., 2000; Kida et al., 2002; Bozon et al., 2003; Kelly et al., 2003); other molecules necessary during the consolidation period do not appear to be necessary during the post-reactivation period (Taubenfeld et al., 2001).

Implications and Conclusions

Our results have strong implications for both basic science and clinical studies of drug addiction. Associations formed between a drug reward and the environment (similar to those formed during conditioned place preference) can impact addicts even after years of abstinence. Further investigation into the post-reactivation lability of conditioned place preference could help to elucidate how these memories are shaped and why they persist for such a long time. Also, Miller and Marshall (2005) recently showed that memories for cocaine CPP can be modulated after their initial formation , and our results confirm that the same general principle holds true for the associations involved in morphine CPP. If similar types of associations can be negatively as well as positively modulated in humans, then a novel avenue of treatment for cue-associated relapse in human addicts could be possible. If drug-environment associations can be negatively modulated in addicts after their initial formation, it may be possible to decrease the chance that exposure to drug-related environments and environmental cues might trigger relapse.

References

Anokhin KV, Tiunova AA, Rose SPR (2002) Reminder effects – reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. *European Journal of Neuroscience*, 15:1759-1765.

Bardo MT, Rowlett JK, Harris MJ (1995) Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neuroscience and biobehavioral reviews*, 19(1):39-51.

Bozon B, Davis S, Laroche S (2003) A requirement for the immediate early gene *zif268* in reconsolidation of recognition memory after retrieval. *Neuron*, 40:695-701.

Brown RW, Bardo MT, Mace DD, Phillips SB, Kraemer PJ (2000) D-Amphetamine facilitation of Morris water task performance blocked by eticlopride and correlated with increased dopamine synthesis in the prefrontal cortex. *Behavioural Brain Research*, 114:135-143.

Cervo L, Mukherjee S, Bertaglia A, Samanin R (1997) Protein kinases A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. *Brain Research*, 775:30-36.

Childress AR, Mozley PD, McElgin W, Fitzeragld J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. *Am J Psychiatry*, 156:11-18.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and

NMDA receptors in the nucleus accumbens. *Proc Natl Acad Sci USA*, 102(17): 6189-94.

Debiec J, LeDoux JE (2004) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience*; 129(2): 267-72.

Dudai Y (1996) Consolidation: fragility on the road to the engram. *Neuron*, 17:367-70.

Dudai Y (2002) Molecular bases of long-term memories: a question of persistence. *Current Opinion in Neurobiology*, 12:211-216.

Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominace. *Science*, 301:1102-1104.

Fenu S, Di Chiara (2003) Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D1 receptors. *Eur J Neurosci*, 18(7):2025-30.

Gaiardi M, Bartoletti M, Gubellini C, Bacchi A, Babbini M (1998) Modulation of the Stimulus Effects of Morphine by *d*-Amphetamine. *Pharmacology, Biochemistry and Behavior*, 59(1): 249-253.

Garavan H, Pankiewicz J, Bloom A, Cho J, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. *Am J Psychiatry*, 157:1789-1798.

Grant S, London ED, Newlin D, Villemagne VL, Lui X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. *Proc. Natl. Acad. Sci. USA*, 93:12040-12045.

Heather N, Stalland A, Tebbutt J (1991) Importance of substance cues in relapse among heroin users: comparison of two methods of investigation. *Addictive Behaviors,* 16:41-49.

Hsu EH, Schroeder JP, Packard MG (2002) The amygdala mediates memory consolidation for an amphetamine conditioned place preference. *Behavioural Brain Research,* 129: 93-100.

Janak PH, Martinez JL Jr (1992) Cocaine and amphetamine facilitate retention of jump-up responding in rats. *Pharmacology, Biochemistry, and Behavior*, 41(4):837-40.

Judge ME, Quartermain D (1982) Characteristics of retrograde amnesia following reactivation of memory in mice. *Physiology and Behavior*, 28:585-590.

Kelly A, Laroche S, Davis S (2003) Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. *Journal of Neuroscience*, 12(23):5354-5360.

Kesner RP, Ellis ME (1983) Memory consolidation: brain region and neurotransmitter specificity. *Neurosci Lett,* 39(3): 295-300.

Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, Silva AJ (2002) CREB required for the stability of new and reactivated fear memories. *Nat Neurosci*, 5(4): 348-355.

Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, Monfils MH, Sutherland RJ, Nader K (2003) Functional organization of adult motor cortex is dependent upon continued protein synthesis. *Neuron*, 40:167-176.

Koh MT, Bernstein IL. Inhibition of protein kinase A activity during conditioned taste aversion retrieval: interference with extinction or reconsolidation of a memory? NeuroReport, 2003; 14(3):405-407

Krivanek JA, McGaugh J (1969) Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. *Agents Actions*, 1(2):36-42.

LaLumiere RT, Buen TV, McGaugh JL (2003) Post-training intra-basolateral amygdala infusions of norepinephrine enhance consolidation of memory for contextual fear conditioning. *J Neurosci*, 23(17): 6754-8.

LaLumiere RT, Nguyen L, McGaugh JL (2004) Post-training intrabasolateral amygdala infusions of dopamine modulate consolidation of inhibitory avoidance memory: involvement of noradrenergic and cholinergic systems. *Eur J Neurosci*, 20(10): 2804-10.

Lee EH, Ma YL (1995) Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. *Brain Res Bull*, 37(4): 411-416.

Lee JLC, Di Chiano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron*, 47: 795-801.

Maas LC, Lukas SE, Kaufman MJ, Weiss RD, Daniels SL, Rogers VW, Kukes TJ, Renshaw PF (1988) Functional magnetic resonance imaging of human brain actiavation during cue-induced cocaine craving. *Am J Psychiatry*, 155(1): 124-126.

McGaugh JL (2000) Memory – a century of consolidation. *Science*, 287:248-251.

Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. *Neuron*, 36:521-525.

Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron*, 47:873-884.

Miranda MI, LaLumiere RT, Buen TV, Bermudez-Rattoni F, McGaugh JL (2003) Blockade of noradrenergic receptors in the basolateral amygdala impairs taste memory. *Eur J Neurosci*, 18(9): 2605-10.

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science*, 160(3827): 554-555.

Nader K, Schafe GE, LeDoux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, 406: 722-26.

Oscos A, Martinez JL Jr., McGaugh JL (1998) Effects of post-training d-amphetamine on acquisition of an appetative autoshaped lever press response in rats. *Psychopharmacology*, 95(1): 132-4.

Pedreira ME, Maldonado H (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron*, 38:863-869.

Phillips GD, Harmer CJ, Hitchcott PK (2002) Blockade of sensitisation-induced facilitation of appetitive conditioning by post-session intra-amygdala nafadotride. *Behav Brain Res*, 134(1-2):249-57.

Przybyslawski J, Sara SJ (1997) Reconsolidation of memory after its reactivation. *Behav Brain Res*, 84:241-246.

Przybyslawski J, Roullet P, Sara SJ (1999) Attenuation of emotional and nonemotional memories after their reactivation: role of β-adrenergic receptors. *J Neurosci*, 19(15):6623-6628.

Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. *Learning and Memory*, 7:73-84.

Schroeder JP, Packard MG (2002) Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. *Behavioral Neuroscience*, 116(5): 922-927.

See RE (2002) Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacology, Biochemistry, and Behavior*, 71:517-529.

Setlow B, McGaugh JL (1998) Sulpiride infused into the nucleus accumbens posttraining impairs memory of spatial water maze training. *Behav Neurosci*, 112(3): 603-610.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*, 168:3-20.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev*, 54(1):1-42.

Sherry JM, Hale MW, Crowe SF (2005) The effects of the dopamine D1 receptor antagonist SCH23390 on memory reconsolidation following reminder-activated retrieval in day-old chicks. *Neurobiol Learn Mem*, 83(2): 104-12.

Shiffman S, Gwaltney CJ, Balabanis MH, Lui KS, Paty JA, Kassel JD, Hickcox M, Gnys M (2002) Immediate antecedents of cigarette smoking: ananalysis from ecological momentary assessment. *J Abnorm Psychol*, 111(4):531-545.

Soetens E, Casaer S, D'Hooge R, Hueting JE (1995) Effect of amphetamine on long-term retention of verbal material. *Psychopharmacology,* 119(2):115-62.

Taubenfeld SM, Milekic MH, Alberini CM (2001) The consolidation of new but not reactivated memory requires hippocampal C/EBPβ. *Nat Neurosci*, 4(8):813-818.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology*, 56:63-672.

Walker MP, Brakefield T, Hobson JA, Stickgold R (2003) Dissociable stages of human meory consolidation and reconsolidation. *Nature*, 425:616-620.

Wolf OT (2003) HPA axis and memory. *Best Pract Res Clin Endocrinol Metab*, 17(2):287-299.

Figure Legends

Figure 1: Effect of immediate post-training injections of amphetamine.

a) Place preference during Test 1 (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score (time spent in morphine-associated chamber minus time spent in saline-associated chamber) ± SEM. (*P<.03)

b) Place preference during Tests 1-5 (vehicle group, n=6; AMPH group, n=6), expressed as a mean difference score ± SEM. A 2-way ANOVA found a main effect of Treatment across all tests (P<.04).

Figure 2: Effect of amphetamine injected immediately after memory reactivation**.**

a) Place preference during Memory Reactivation (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score ± SEM.

b) Place preference during Test 1 (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score ± SEM. (*P<.04)

e) Place preference during Tests 1-3 (vehicle group, n=6; AMPH group, n=6), expressed as a mean difference score ± SEM. A 2-way ANOVA found a main effect of Treatment across all tests (P<.05).

Figure 3: Effect of amphetamine injected immediately vs. 6 hours after training.

Place preference during the Test session (AMPH-immediately group, n=15; AMPH-6 hrs group, n=15), expressed as a mean difference score ± SEM. (*P<.02)

Figure 4: Effect of amphetamine injected immediately vs. 6 hours after memory reactivation.

a) Place preference during Memory Reactivation (AMPH-immediately group, n=11; AMPH-6 hrs group, n=11), expressed as a mean difference score ± SEM.

b) Place preference during Test 1 (AMPH-immediately group, n=11; AMPH-6 hrs group, n=11), expressed as a mean difference score ± SEM. (*P<.02)

Table 1: The mean amount of time in seconds (± SEM) spent in the morphine-paired and saline-paired chambers by each group for each experiment. The times spent in the morphine-paired versus the saline-paired chambers were compared using paired *t*-tests, and the resulting P-values are listed in the last column.

Figure 1

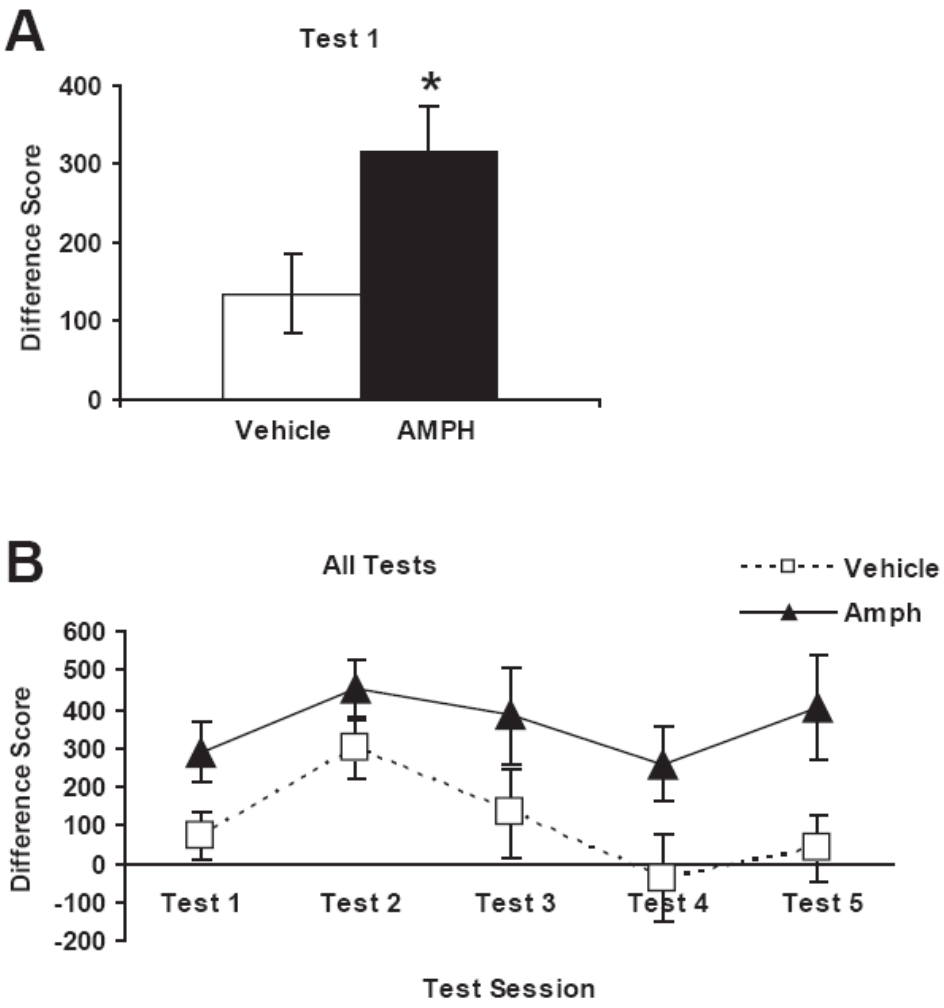

A
Test 1
*
400
300
200
100
0
Difference Score
Vehicle
AMPH
B
All Tests
Vehicle
Amph
600
500
400
300
200
100
0
-100
-200
Difference Score
Test 1
Test 2
Test 3
Test 4
Test 5
Test Session

Figure 2

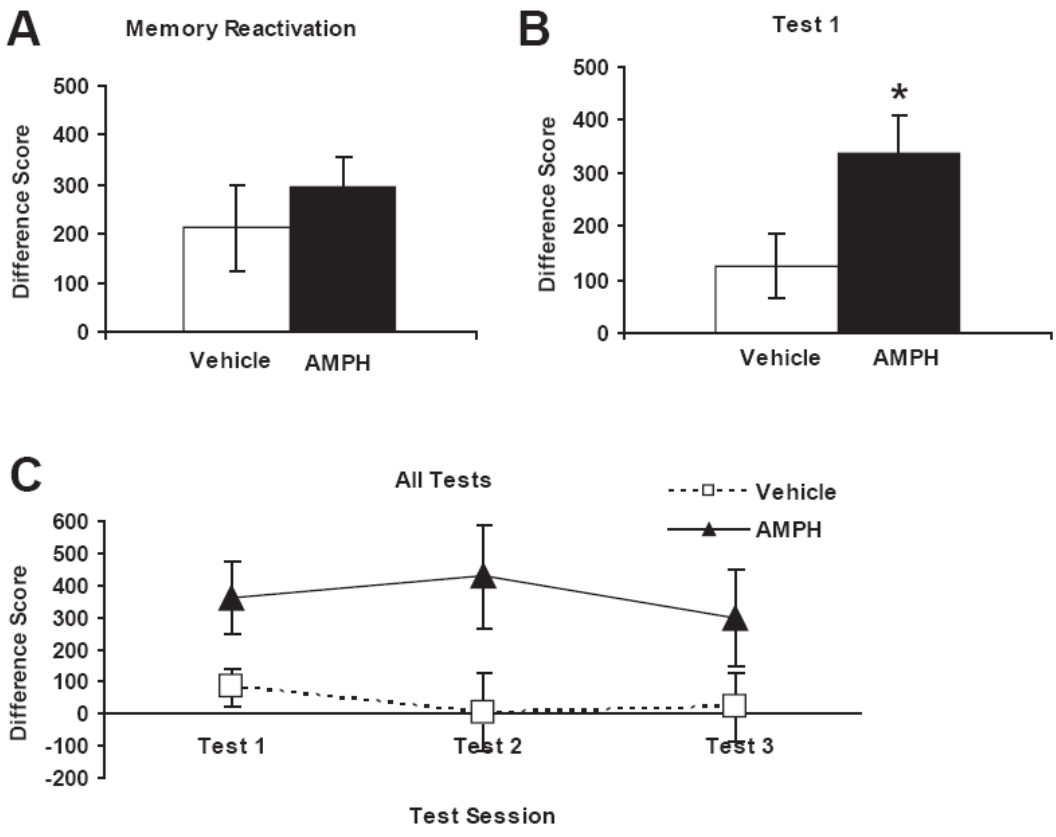

A
Memory Reactivation
Difference Score
500
400
300
200
100
0
Vehicle
AMPH
B
Test 1
Difference Score
500
400
300
200
100
0
*
Vehicle
AMPH
C
All Tests
Vehicle
AMPH
Difference Score
600
500
400
300
200
100
0
-100
-200
Test 1
Test 2
Test 3
Test Session

Figure 3

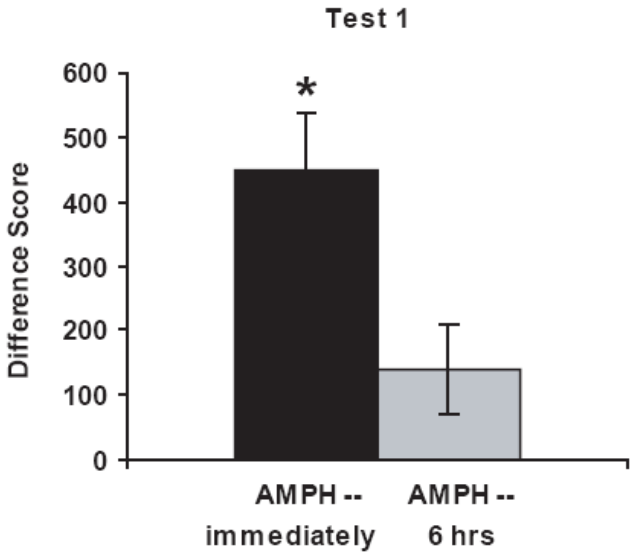

Test 1
Difference Score
600
500
400
300
200
100
0
*
AMPH -- immediately
AMPH -- 6 hrs

Figure 4

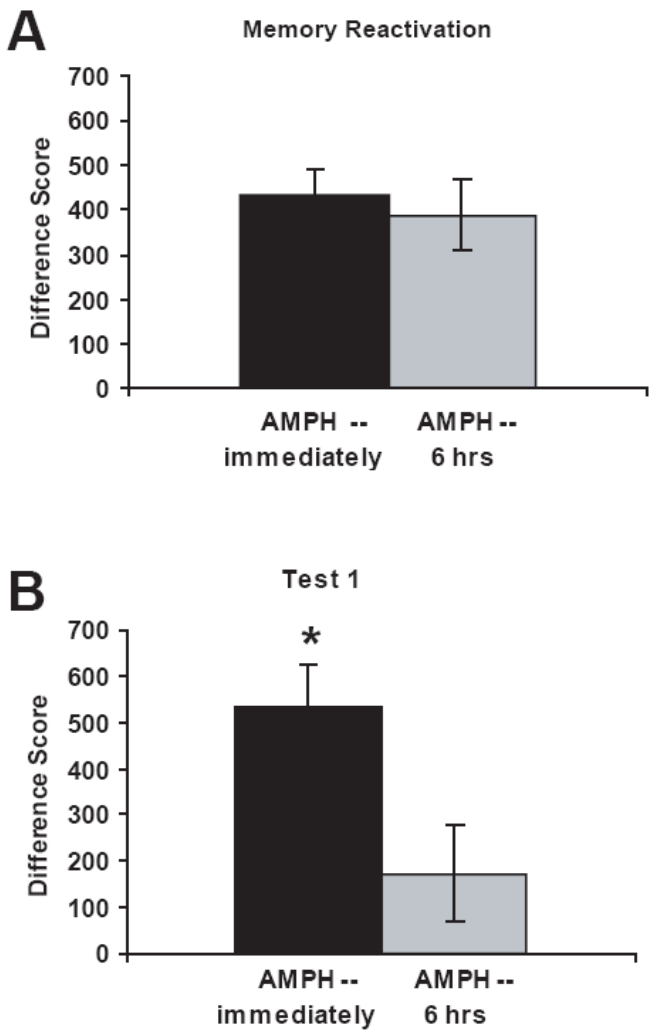

A
Memory Reactivation
Difference Score
700
600
500
400
300
200
100
0
AMPH -- immediately
AMPH -- 6 hrs
B
Test 1
*
Difference Score
700
600
500
400
300
200
100
0
AMPH -- immediately
AMPH -- 6 hrs

Table 1

Experiment	Test Session	Group	Mean (±SEM) time in morphine chamber (sec)	Mean (±SEM) time in saline chamber (sec)	P-value
1a	Test 1	Veh	722.76 (± 30.24)	587.92 (± 25.61)	<.02
		Amph	768.11 (± 29.97)	451.71 (± 36.80)	<.001
1b	Memory Reactivation	Veh	693.03 (± 36.31)	481.86 (± 60.72)	<.008
		Amph	724.1 (± 38.04)	427.33 (± 31.98)	<.001
	Test 1	Veh	606.07 (± 45.09)	480.28 (± 45.55)	0.07
		Amph	739.69 (± 50.61)	404.17 (± 32.19)	<.001
2a	Test 1	Amph - imm	870.37 (± 52.36)	420.27 (± 40.33)	<.001
		Amph - 6 hrs	718.42 (± 31.00)	579.39 (± 43.85)	0.095
2b	Memory Reactivation	Amph - imm	833.73 (± 37.20)	398.52 (± 32.46)	<.001
		Amph - 6 hrs	831.1 (± 51.35)	442.72 (± 46.39)	<.001
	Test 1	Amph - imm	917.68 (± 56.87)	385.67 (± 41.23)	<.001
		Amph - 6 hrs	731.43 (± 51.50)	559.2 (± 55.26)	0.098
3	Test 1	Veh	772.02 (± 59.67)	357.92 (± 36.04)	<.001
		Amph	831.48 (± 60.37)	366.23 (± 37.16)	<.001
4	Test 1	Veh	732.72 (± 80.74)	568.49 (± 73.57)	.32
		Amph	673.99 (± 66.29)	540.28 (± 68.09)	.34

Chapter Three:

Post-training, but not Post-reactivation, Administration of Amphetamine and Anisomycin Modulates Pavlovian Conditioned Approach

Abstract

The psychostimulant, amphetamine (AMPH), and the protein synthesis inhibitor, anisomycin (ANI), have been shown to modulate the consolidation and reconsolidation of several types of learning. To determine whether Pavlovian conditioned approach (PCA) is modulated in a similar manner, we examined the effects of post-training and post-reactivation administration of both AMPH and ANI on memory for PCA. Male Long-Evans rats received PCA training sessions during which presentations of a CS+ were followed by sucrose delivery. AMPH (1 mg/kg, s.c.) injected immediately but not 6 hrs after the first training session enhanced PCA behavior. ANI (150 mg/kg, s.c.) injected immediately but not 3 hrs after the first training session impaired PCA behavior. This impairment was not due to the development of a conditioned taste aversion. To examine whether PCA can also be modulated by post-reactivation administration of AMPH and ANI, rats were given an injection of AMPH, ANI, or vehicle immediately after a memory reactivation session. Upon testing, the behavior of both the AMPH- and the ANI-treated rats was unaffected. This result remained consistent when the experiment was repeated with changes to various behavioral parameters (i.e. amount of training, length of memory

reactivation). These findings indicate that AMPH and ANI act during the post-training but not the post-reactivation period to enhance and impair, respectively, the learning of PCA. This suggests that the consolidation of PCA can be modulated in a manner comparable to other types of learned associations, but once learned, the memory appears to be relatively robust and stable.

Introduction

Consolidation refers to a process that occurs immediately after the initial acquisition of a memory during which the long-term memory is thought to be forming and stabilizing. It was first observed by neurologists at the turn of the last century, and it has since been observed across all types of memories and species that have been investigated (McGaugh, 2000; Sara, 2000; Dudai, 2004). While the consolidation process is ongoing, memory is labile, and it can be both positively and negatively modulated by a variety of pharmacological agents (Abel and Lattal, 2001; Dudai, 2004).

Two pharmacological agents that reliably modulate the consolidation of multiple types of memories are amphetamine (AMPH), a psychostimulant, and anisomycin (ANI), a drug that inhibits the synthesis of new proteins by blocking translation. It has been known for many years that psychostimulants can enhance consolidation in a variety of learning tasks (McGaugh and Petrinovich, 1965; McGaugh, 1966, 2002). Systemic injections of AMPH have been shown to enhance the consolidation of aversive tasks such as conditioned taste aversion (Fenu and Di Chiara, 2003) and various types of avoidance learning (Doty and Doty, 1966; Kulkarni, 1968; Haycock et al., 1977; Martinez et al., 1980; Janak and Martinez, 1992). In addition, systemic injections of AMPH have been

shown to enhance the consolidation of spatial learning (Packard and White, 1989; Strupp et al., 1991; Brown et al., 2000), visual discrimination learning (Krivanek and McGaugh, 1969), and appetitive conditioning (Oscos et al., 1988; Simon and Setlow, 2006). Systemic injections of AMPH can also enhance the consolidation of verbal learning in humans (Soetens et al., 1993; Soetens et al., 1995). In contrast, ANI has been shown to impair the consolidation of multiple, different memory tasks (Davis and Squire, 1984). For example, ANI can impair consolidation in tasks such as conditioned taste aversion (Rosenblum et al., 1993), discrimination learning, (Squire and Barondes, 1974; Squire and Davis, 1975), fear conditioning (Schafe and LeDoux, 2000; Epstein et al., 2003), spatial memory (Meiri and Rosenblum, 1998), and instrumental conditioning (Hernandez et al., 2002).

Both AMPH and ANI have also been shown to modulate memories when they are administered immediately after memory reactivation (Alberini, 2005; Dudai, 2006). For example, post-reactivation administration of AMPH has been shown to enhance morphine conditioned place preference (Blaiss and Janak, 2006), and post-reactivation administration of ANI has been shown to impair memory in tasks such as cued fear conditioning and conditioned taste aversion (Judge and Quartermain, 1982; Nader et al., 2000a; Eisenberg et al., 2003). It is hypothesized that post-reactivation administration of such drugs is able to affect memories by modulating a process of re-stabilization (termed reconsolidation) that occurs after memory retrieval and is required for maintenance of the memory.

The mechanisms underlying the consolidation and maintenance of appetitive Pavlovian conditioning tasks, such as Pavlovian conditioned approach (PCA), have not

been widely studied. In the PCA task, the presentation of an initially neutral stimulus (CS+) predicts the subsequent delivery of a reward (US). As subjects learn to associate the CS+ with the US, they begin to show enhanced reward-seeking behavior during the CS+. In this study, we tested the effect of a single injection of AMPH or ANI on the early consolidation of PCA. In addition, it is not known whether the cue-reward associations formed during the PCA task exhibit any sort of lability after reactivation of the memory; therefore, we also investigated the ability of either AMPH or ANI to modulate the PCA memory when administered after memory reactivation.

Materials and Methods

Subjects

Male Long-Evans rats (Harlan, Indianapolis, IN, USA) weighing between 200-350g were individually housed in polycarbonate cages. Subjects were kept on a 12-h light:12-h dark cycle (lights on at 7 a.m.), and all behavioral procedures were conducted during the light cycle. Rats were water restricted beginning the day before the start of behavioral training; they were allowed free access to water for two hours per day, immediately after behavioral sessions. All rats received food ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center at the University of California, San Francisco, and are in accordance with "PHS Policy on Humane Care and Use of Laboratory Animals," Office of Laboratory Animal Welfare, National Institutes of Health, USA, revised 2002.

Drugs

D-Amphetamine (Sigma, St. Louis, MO, USA) was dissolved in physiological sterile saline and injected at a dose of 1 mg/kg. This dose of amphetamine was chosen based on its ability to enhance memory consolidation in a variety of behavioral paradigms (Kulkarni, 1968; Krivanek and McGaugh, 1969; Haycock et al., 1977; Martinez et al., 1980; Oscos et al., 1988; Packard and White, 1989; Janak and Martinez, 1992; Brown et al., 2000; Fenu and Di Chiara, 2003; Blaiss and Janak, 2006; Simon and Setlow, 2006). Anisomycin (Sigma) was initially dissolved in 1N HCl, then diluted in physiological sterile saline, and finally adjusted to a pH of 7.2. Anisomycin was injected at a dose of 150 mg/kg. This dose was chosen because of its ability to impair memory consolidation and reconsolidation in other behavioral paradigms (Davis and Squire, 1984; Taubenfeld et al., 2001). In one experiment, anisomycin was injected at a dose of 75 mg/kg. This dosage change is noted in the text. All injections were administered subcutaneously at an injection volume of 1 mL/kg.

Apparatus

Conditioning chambers (Med Associates Inc., Georgia, VT, USA) were enclosed in larger sound attenuating cubicles. Syringe pumps delivered sucrose solution into a fluid receptacle located in a rectangular recess (sucrose port) in the right wall of the chamber, and photocells detected entries into the sucrose port. A white stimulus light and a houselight were located on the right wall of the chamber, and a 2.9 kHz tone could be played through speakers also located on the right wall of the chamber. A computer with

Med Associates software controlled all behavioral equipment and recorded entries into the sucrose port.

Behavioral Procedures

Sucrose Port Training.

Rats were given one session of sucrose port training to learn to consume sucrose from the delivery alcove. After a 5 min habituation period, 30 deliveries of a 10% sucrose solution (.2 mL; w/v, in filtered water) were presented on a VI-120 sec schedule.

Pavlovian Conditioned Approach (PCA) Training Sessions.

Rats received one PCA training session per day. During each session, there was an initial 5 min habituation period before the start of training trials. Each training trial consisted of a 10 sec presentation of either a CS+ or CS-. The stimuli used were a tone and a lighting change (the stimulus light turned on while the houselight was simultaneously turned off), and the choice of stimulus to be used as the CS+ was counterbalanced across subjects. There were 15 presentations of each type of CS, delivered in random order on a VI-150 sec schedule. Presentation of the CS+ was immediately followed by the delivery of a 10% sucrose solution (.2 mL; w/v, in filtered water) through a syringe pump. Each training session lasted for a total of 90 minutes.

Memory Reactivation and Subsequent Test Sessions.

The memory reactivation and subsequent test sessions were identical to the training sessions, with two critical differences. There were only 5 presentations each of the CS+ and CS-, and no sucrose was delivered. In two experiments, memory reactivation sessions were different from the test sessions due to changes made to the memory

reactivation session. For the experiment with a rewarded reactivation session, the memory reactivation session included delivery of the 10% sucrose solution immediately following all presentations of the CS+. For the experiment with a short reactivation session, the session had only 1 presentation each of the CS+ and the CS-.

Sucrose Preference Test.

On the two days when animals were tested for a conditioned taste aversion to sucrose, animals remained in their home cages. They were given two hour daily access to two bottles of liquid (one containing filtered water and one containing the same 10% sucrose solution used in the PCA training sessions), and their consumption was measured. The spatial location of the bottles was switched between days to control for the possibility of a spatial bias. The two sucrose preference tests occurred during the same time of day when rats would normally be experiencing PCA training sessions. To calculate a sucrose preference ratio, we divided the volume of sucrose consumed by the total volume of liquid consumed.

Data Analysis

During PCA sessions, the number of port entries into the sucrose port during the CS+ and CS- were recorded by computer. To calculate a measure of learning that compared responding during the CS+ with responding during the CS-, we subtracted the number of port entries made during the CS- from the number of port entries made during the CS+. We termed this the difference score. The data were initially analyzed using 2-way repeated measures ANOVAs, with Treatment as a between-subjects factor and Bin, Day, or Session as a within-subjects factor. Significant main effects and/or interactions were

followed by planned comparisons. All statistical analyses were conducted using SigmaStat (SPSS, Inc., Chicago, IL, USA).

Results

Post-training Administration of Amphetamine Enhances Pavlovian Conditioned Approach

To examine the effect of AMPH on the consolidation of PCA, we tested whether immediate post-training injections of AMPH would enhance learning of PCA. Because the consolidation process occurs during a limited time window following training, AMPH should no longer modulate learning when injected several hours after training. Therefore, we also examined the effect of AMPH when injected 3 hrs after training. Because animal handling and subcutaneous injections of vehicle can modulate consolidation (Hui et al., 2006), we designed this experiment to control for the possibility that injections (and the related handling) occurring at different times in different groups could act as a confounding factor. Subjects were first given one PCA training session, and then all animals were given two injections: one immediately after the training session and one 3 hrs after the training session (see Figure 1a for an outline of these methods). The first group (veh; n=15) received injections of vehicle both immediately and 3 hrs after training; the second group (AMPH – imm; n=12) received an injection of AMPH immediately after training and an injection of vehicle 3 hrs after training; and the third group (AMPH – 3 hrs; n=7) received an injection of vehicle immediately after training and an injection of AMPH 3 hrs after training. Subjects then experienced a second PCA training session 24 hrs after the first one.

When the data were summarized for training session 2, the immediate post-training injections of AMPH did not appear to have enhanced PCA behavior (see Figure 1b). A two-way repeated measures ANOVA analyzing the difference scores across training sessions did not find a significant effect of treatment or a significant interaction [Treatment: $F(2,31)=.38$; $P=.68$; Training Session: $F(1,31)=55.29$; $P<.001$; Interaction: $F(2,31)=.95$; $P=.40$]. However, it was possible that the enhancing effect of AMPH was obscured by within-session learning in the vehicle group during training session 2.

If immediate post-training injections of AMPH truly enhanced consolidation (the learning that occurred between training sessions 1 and 2), then the memory enhancing effect of AMPH should be most apparent during the early trials of the second training session. If the vehicle group showed a significant amount of within-session learning during the later trials of that session, any enhancing effect of AMPH seen during the early trials of the session would simply be washed out when the data from the session were summarized. To see whether this was the case, the data from training session 2 were analyzed in three bins of five trials each (see Figure 1c), and a two-way repeated measures ANOVA found a significant main effect of Bin and a significant interaction between Bin and Treatment [Treatment: $F(2,31)=.93$, $P=.41$; Bin: $F(2,31)=5.27$, $P<.008$; Interaction: $F(4,31)=3.34$, $P<.02$]. Further analysis showed that during the 1st bin (trials 1-5), animals that received immediate injections of AMPH exhibited a greater difference score compared to animals that received vehicle [unpaired t-test, $t(25)=-2.21$; $P<.04$]. In addition, as we hypothesized, the animals that received vehicle injections did show a significant amount of within-session learning during this session. Within the vehicle group, the difference score increased between the 1st and 2nd bin [paired t-test, $t(14)=-$

2.66, P<.02] and between the 2nd and 3rd bin [paired t-test, t(14)=-2.33, P<.04]. Therefore, immediate post-training injections of AMPH did enhance memory for PCA, but when the data from training session 2 were summarized, the vehicle group's within-session learning obscured this effect.

Because consolidation occurs during a limited time window, delayed post-training injections of AMPH should not enhance behavior for PCA. In keeping with this hypothesis, animals that received injections of AMPH 3 hrs after training (unlike those that received immediate post-training injections of AMPH) did not show enhanced PCA behavior during the 1st bin of training session 2 when compared to the vehicle group [see Figure 1c; unpaired t-test, t(20)=-.65, P=.52]. During this bin, however, the difference score of the animals that received AMPH 3 hrs after training was not significantly different from the difference score of the animals that received AMPH immediately after training [unpaired t-test, t(17)=1.33, P=.20]. This suggests that, although they did not have the same effect as immediate post-training injections of AMPH, injections of AMPH 3 hrs after training had a slight enhancing effect on the consolidation of PCA. Therefore, we hypothesized that the consolidation process was still ongoing 3 hrs after training. We should note that in Figure 1c, the mean difference score of the animals that received AMPH 3 hrs after training is lower than the mean difference score of the other groups during the 3rd bin (Trials 11-15) of training session 2. However, there is no significant difference between groups during this bin [one-way ANOVA; Treatment: $F(2,31) = 1.92$; $P = .164$]. Inspection of the data revealed that the lower mean was due to one subject.

To determine whether the memory remained sensitive to AMPH at a time point later than 3 hrs after training, we conducted another experiment (see Figure 2a for a general description of the methods). Subjects were first given one PCA training session. They were injected with either vehicle (n=7) or AMPH (n=8) 6 hrs after this training session, and then they experienced a second training session 24 hrs after the first one. When the data for training session 2 were summarized, there was no difference between the groups [see Figure 2b; 2-way repeated measures ANOVA; Treatment: $F(1,13)=.22$, $P=.65$; Training Session: $F(1,13)=9.47$, $P<.009$; Interaction: $F(1,13)=.39$, $P=.54$]. Figure 2c shows the data from training session 2 separated into 3 bins of five trials each, and when the data were analyzed in this manner, there was again no difference between rats that received vehicle or AMPH 6 hrs after training [2-way repeated measures ANOVA; Treatment: $F(1,13)=.28$, $P=.60$; Bin: $F(2,13)=6.06$, $P<.007$; Interaction: $F(2,13)=.002$, $P=.998$]. Therefore, injections of AMPH 6 hrs after training had no effect on PCA behavior, suggesting that the memory had fully consolidated by this time point.

Post-training Administration of Anisomycin Impairs Pavlovian Conditioned Approach

In order to investigate the effect of ANI on the consolidation of PCA, subjects were given one PCA training session, and then all animals were given two injections: one immediately after the training session and one 3 hrs after the training session. The first group (veh; n=15) received injections of vehicle both immediately and 3 hrs after training; the second group (ANI – imm; n=7) received an injection of ANI immediately after training and an injection of vehicle 3 hrs after training; and the third group (ANI – 3

hrs; n=7) received an injection of vehicle immediately after training and an injection of ANI 3 hrs after training. Subjects then experienced a second PCA training session 24 hrs after the first one (see Figure 3a for an outline of these methods).

Rats that received immediate (but not delayed) post-training injections of ANI exhibited an impaired ability to discriminate between the CS+ and CS- during training session 2 (see Figure 3b). A two-way repeated measures ANOVA analyzing the difference scores across training sessions found a significant main effect of Training Session and a significant interaction between Treatment and Training Session [Treatment: $F(2,26)=2.86$, $P=.09$; Training Session: $F(1,26)=19.82$, $P<.001$; Interaction: $F(2,26)=4.18$, $P<.03$]. Further analysis showed that the group that received immediate post-training injections of ANI was significantly impaired during training session 2 when compared to the vehicle group [unpaired t-test, $t(20)=3.41$, $P<.003$]. In contrast to the immediate injections, injections of ANI 3 hrs after training had no effect on PCA behavior. During training session 2, the behavior of this group was not significantly different from the vehicle group [unpaired t-test, $t(20)=.01$, $P=.989$], but it was significantly different when compared to the group that received immediate injections of ANI [unpaired t-test, $t(12)=-3.27$, $P<.007$]. In addition, while the difference scores of both the vehicle group and the ANI – 3hrs group increased between training sessions 1 and 2 [veh group: paired t-test, $t(14)=-4.64$, $P<.001$; ANI – 3 hrs group: paired t-test, $t(6)=-3.31$, $P<.02$], the ANI – imm group did not exhibit any between-session learning [paired t-test, $t(6)=-.19$, $P=.85$]. This suggests that, while post-training injections of ANI impaired PCA, the window of vulnerability to ANI closed within 3 hrs of training.

We next investigated whether ANI has a dose-dependent effect on PCA consolidation. The general methods for this experiment are described in Figure 3c. Subjects were first given one PCA training session, and immediately afterwards, they were injected with either vehicle (n=8) or a low dose of ANI (75 mg/kg; n=8). During the second training session the next day, there was no difference between the two groups (see Figure 3d). A two-way repeated measures ANOVA analyzing the difference scores across training sessions found no main effect of Treatment [$F(1,14)=.81$, $P=.39$] and no significant interaction between Treatment and Training Session [$F(1,14)=.52$, $P=.48$]. However, there was a significant main effect of Training Session [$F(1,14)=22.70$, $P<.001$], and further analysis showed that the difference scores of both the vehicle group [paired t-test, $t(7)=-3.87$, $P<.006$] and the ANI group [paired t-test, $t(7)=-3.22$, $P<.02$] increased significantly between training sessions 1 and 2. This suggests that the 75 mg/kg dose of ANI did not impair the consolidation of PCA.

Because systemic injections of ANI can cause temporary illness in rats (we observed piloerection, a hunched back posture, and weight loss), it was important to confirm that the impairment in PCA behavior seen in subjects with post-training ANI injections (see Figure 3b) was due to an impairing effect of the drug on the consolidation of the memory. It was also possible that these rats exhibited impaired PCA behavior because they developed an ANI-induced conditioned taste aversion to sucrose and were no longer motivated to consume it. We tested this possibility directly with a separate experiment (see Figure 4a for a general outline of the methods). Subjects were first given one PCA training session, and immediately afterwards, they were injected with either vehicle (n=8) or ANI (150 mg/kg, s.c., n=8). During the next two days, rats were given

two sucrose preference tests in order to determine whether they had developed a conditioned taste aversion to sucrose. They were allowed access to liquids (one bottle of water and one bottle of sucrose) for two hours per day (food was provided ad libitum), and their consumption was measured. Figure 4b shows the sucrose preference ratio across both days of sucrose preference tests. All subjects preferred sucrose over water, and there was no difference in preference level between the vehicle group and the ANI group [2-way repeated measures ANOVA; Treatment: $F(1,14)=.211$, $P=.65$; Day: $F(1,14)=1.15$, $P=.30$; Interaction: $F(1,14)=1.08$, P.32], suggesting that injections of ANI did not cause rats to develop a conditioned taste aversion to sucrose. Twenty-four hours after the last sucrose preference test, subjects were given a second PCA training session. A two-way repeated measures ANOVA analyzing the difference scores across training sessions found a significant main effect of Training Session and a significant interaction between Treatment and Training Session [Treatment: $F(1,14)=1.45$, $P=.25$; Training Session: $F(1,14)=36.35$, $P<.001$; Interaction: $F(1,14)=4.96$, $P<.04$]. Further analysis found that during the second training session, the ANI group exhibited an impaired ability to discriminate between the CS+ and CS- when compared to the vehicle group [see Figure 4c; unpaired t-test, $t(14)=2.4$, $P<.03$]. This suggests that immediate post-training injections of ANI impaired the consolidation of PCA, and the impairment in behavior was not due to the development of a conditioned taste aversion to the US.

Post-reactivation Administration of Anisomycin Does Not Affect Pavlovian Conditioned Approach

To determine whether post-reactivation administration of ANI would also impair PCA behavior, rats were first given two PCA training sessions, and the next day they were given a memory reactivation session. The memory reactivation session was similar to the training sessions, but there were two critical differences: it was only 30 minutes long (5 trials instead of the 15 trials during training sessions), and no sucrose was delivered. Immediately after the memory reactivation session, subjects were injected with either vehicle (n=19) or ANI (n=20). The next day, rats experienced the first of two test sessions (separated by 24 hrs). The test sessions were identical to the memory reactivation session (see Figure 5a for a general outline of these methods). Figure 5b shows the difference scores of both groups during the memory reactivation and test sessions. Although a two-way repeated measures ANOVA did find a significant amount of extinction across sessions [Session: $F(2,37)=28.30$, $P<.001$], there was no difference between rats that received ANI and those that received vehicle [Treatment: $F(1,37)=.20$, $P=.66$; Interaction: $F(2,37)=.75$, $P=.48$]. This suggests that post-reactivation administration of ANI did not affect the continued stability of the original PCA memory.

However, in other memory tasks, the strength of training in the task can alter the effect of post-reactivation injections of ANI on behavior. When the number of trials in the training session for a contextual fear conditioning task was increased, the resulting memory became less vulnerable to the impairing effect of an injection of ANI given immediately before reactivation (Suzuki et al., 2004). In contrast, when the number of training trials for a conditioned taste aversion task was increased, the resulting memory

became more vulnerable to the impairing effect of a post-reactivation injection of ANI (Eisenberg et al., 2003). While the effect of training strength on the post-reactivation lability of memories is not well understood at this time, these studies do indicate that training strength can alter the effect of post-reactivation injections of ANI. Therefore, we repeated our previous experiment, but we increased the amount of training given to the rats from 2 PCA training sessions to 4 or 7 PCA training sessions (see Figure 5a for general methods). They were then given a memory reactivation session 24 hrs later, and immediately after this session, they received an injection of vehicle (4 training sessions: n=8; 7 training sessions: n=8) or ANI (4 training sessions: n=8; 7 training sessions: n=8). All subjects then experienced test sessions beginning 24 hrs later. The difference scores for the rats that received 4 trainings sessions are shown in Figure 5c. A two-way repeated measures ANOVA analyzing the difference scores across sessions for subjects receiving 4 training sessions found no difference between groups [Treatment: $F(1,14)=.16$, $P=.70$; Session: $F(2,14)=5.51$, $P<.01$; Interaction: $F(2,14)=.59$, $P=.56$]. The same was true for rats that received 7 training sessions [Figure 5d; 2-way repeated measures ANOVA; Treatment: $F(1,14)=1.11$, $P=.31$; Session: $F(2,14)=30.64$, $P<.001$; Interaction: $F(2,14)=.93$, $P=.41$]. Therefore, increasing the number of training sessions did not uncover any sensitivity to post-reactivation injections of ANI.

However, the length of the reactivation session may also alter the ability of post-reactivation injections of ANI to impair the original memory. For example, experiments conducted in mice, fish, and crabs showed that when the memory reactivation session was brief enough that it produced limited extinction learning, post-reactivation administration of protein synthesis inhibitors impaired the original memory, but when the

reactivation session was long enough to produce considerable extinction learning, post-reactivation administration of protein synthesis inhibitors no longer had the same effect (Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Suzuki et al., 2004). Therefore, we altered the memory reactivation session in two different ways in order to decrease the amount of extinction learning during the reactivation (see Figure 6a for an outline of the general methods for these experiments). For the first experimental variation, subjects were given 2 training sessions. The next day, there was a memory reactivation session, and although this session was the same length as previous reactivation sessions (5 trials), sucrose was delivered immediately following presentation of the CS+. In other words, this session was no longer an extinction session. Immediately after the reactivation session, rats were injected with vehicle (n=8) or ANI (n=8), and they were then given two tests over the next two days. The difference scores during the reactivation session and the two test sessions are shown in Figure 6b. A two-way repeated measures ANOVA analyzing the difference scores across all sessions found no difference between groups [Treatment: $F(1,14)=1.54$, $P=.24$; Session: $F(2,14)=27.69$, $P<.001$; Interaction: $F(2,14)=.63$, $P=.54$].

In the second experimental variation, subjects were first given 4 training sessions. The number of training sessions was increased to 4 because increasing the number of training trials can render a memory more resistant to extinction (Eisenberg et al., 2003). The next day, there was a memory reactivation session. This session was conducted in extinction (no sucrose was delivered), and there was only 1 trial (one presentation of the CS+ and one presentation of the CS-, in random order) instead of the usual 5 trials. Immediately after the reactivation session, rats were injected with vehicle (n=8) or ANI

(n=8), and they were then given two tests over the next two days. The difference scores during the reactivation session and the two test sessions are shown in Figure 6c. As in the previous experiment, a two-way repeated measures ANOVA analyzing the difference scores across all sessions found no difference between groups [Treatment: $F(1,14)=.17$, $P=.68$; Session: $F(2,14)=6.34$, $P<.005$; Interaction: $F(2,14)=.75$, $P=.48$]. Therefore, even with multiple, different alterations in the behavioral parameters, post-reactivation injections of ANI did not affect PCA behavior.

Post-reactivation Administration of Amphetamine Does Not Affect Pavlovian Conditioned Approach

To determine the effect of post-reactivation injections of AMPH on PCA behavior, we used an experimental design similar to the experiments examining the effect of post-reactivation administration of ANI on PCA (described above). Rats were first given PCA training sessions (either 2 or 7), and 24 hrs after the last training session, they experienced a memory reactivation session (5 trials with no sucrose delivery). Immediately after the reactivation session, rats were injected with vehicle (2 training sessions: n=8; 7 training sessions: n=8) or AMPH (2 training sessions: n=8; 7 training sessions: n=8) and given a test 24 hrs later (see Figure 7a for an outline of the general methods). The difference scores for the rats that received 2 trainings sessions are shown in Figure 7b. Although a two-way repeated measures ANOVA found that there was a significant amount of extinction across sessions [Session: $F(2,14)=19.79$, $P<.001$], there was no difference between rats that received AMPH and those that received vehicle [Treatment: $F(1,14)=.02$, $P=.89$; Interaction: $F(2,14)=.13$, $P=.88$]. The same was true for

rats that received 7 training sessions (see Figure 7c). A two-way repeated measures ANOVA found that there was a significant amount of extinction across sessions [Session: $F(2,14)=11.50$, $P<.004$], but there was no difference between rats that received AMPH and those that received vehicle [Treatment: $F(1,14)=.17$, $P=.69$; Interaction: $F(2,14)=.53$, $P=.48$]. Therefore, even with alterations in the training parameters, post-reactivation injections of AMPH did not modulate PCA behavior.

Discussion

These experimental findings show that AMPH injected immediately, but not 6 hrs, after training enhanced the consolidation of PCA. In addition, we have shown that ANI injected immediately, but not 3 hrs, after training impaired the consolidation of PCA. ANI injections did not cause the rats to develop a conditioned taste aversion to the US, suggesting that the ANI-induced behavioral impairment was truly an effect on memory. These results indicate that the consolidation of the associations learned in PCA can be modulated in a manner comparable to other types of learned associations.

We have also shown that ANI and AMPH had no effect on PCA behavior when administered immediately after memory reactivation, and this result was consistent even when the behavioral design was varied in ways that could affect the strength of the memory. These results suggest that post-reactivation administration of AMPH and ANI does not modulate the original PCA memory. In this respect, PCA differs from several other types of memory tasks, such as fear conditioning, inhibitory avoidance, conditioned taste aversion, and conditioned place preference (Nader et al., 2000b; Milekic and Alberini, 2002; Eisenberg et al., 2003; Blaiss and Janak, 2006).

Post-training Administration of Amphetamine and Anisomycin

Amphetamine injected immediately after the 1st training session resulted in an increased ability of subjects to discriminate between the CS+ and CS- during the early trials of the 2nd training session. Injections of AMPH 3 hrs after the 1st training session produced a mild, non-significant enhancement of behavior, but injections of AMPH 6 hrs after the 1st training session produced no behavioral enhancement. This suggests that, for the PCA memory, the consolidation process is completed some time between 3 and 6 hrs after training. While it has been shown previously that multiple post-training injections of AMPH enhance PCA learning (Hitchcott et al., 1997b; Phillips et al., 2003; Simon and Setlow, 2006), the findings described above show that a single post-training injection of AMPH enhances the early consolidation of this task.

Further studies are needed to determine the mechanisms by which post-training injections of AMPH enhance PCA. Experiments using other types of learning and memory tasks have investigated the mechanisms underlying the memory enhancing effects of amphetamine and have implicated both the norepinephrine and dopamine systems (Lee and Ma, 1995; Brown et al., 2000; Phillips et al., 2002), and it is possible that AMPH is acting through these neurotransmitter systems to produce our results.

In contrast to the effect of AMPH, injections of ANI immediately (but not 3 hrs) after the 1st training session resulted in an impaired ability of subjects to discriminate between the CS+ and CS- during the 2nd training session, suggesting that ANI impaired the early consolidation of this task. Immediate post-training injections of a low dose of

ANI had no effect on behavior, but this dose does produce symptoms of illness in animals (Hernandez and Kelley, 2004). Therefore, it is unlikely that behavioral impairment caused by immediate post-training injections of ANI was purely the result of ANI-induced illness.

To test this possibility more directly, we investigated whether immediate post-training injections of ANI resulted in the development of a conditioned taste aversion to the US (a sucrose solution). We found that ANI-treated animals did not develop a conditioned taste aversion, and in fact, they showed a strong preference for the US. However, in a 2nd PCA training session, they still exhibited an impaired ability to discriminate between the CS+ and the CS-. These findings indicate that immediate post-training injections of ANI impaired the consolidation of the PCA memory, and the behavioral impairment cannot be explained as a generalized side-effect of ANI. Because ANI is an inhibitor of translation, these findings suggests that ANI impairs consolidation by impairing the production of new proteins required for the formation of the PCA memory.

Further studies will help to determine the specific brain regions where ANI and AMPH act to modulate the early consolidation of PCA. Experiments administering infusions over multiple trials have shown that AMPH can act within the amygdala or the nucleus accumbens shell to enhance the acquisition of appetitive Pavlovian conditioning (Hitchcott et al., 1997b; Phillips et al., 2003; Dalley et al., 2005), but AMPH could also be acting in additional areas. Although it is possible that both AMPH and ANI are acting in the same brain region, the specific sites of action need not be the same. For example,

AMPH may act in one brain region to modulate plasticity that is actually occurring in a separate region.

To date, studies have implicated a general cortico-limbic-striatal circuit in the acquisition of several varieties of appetitive Pavlovian conditioning. In rats, neurons in the amygdala selectively increase their firing during the presentation of conditioned stimuli (both auditory and visual) that predict sucrose delivery (Toyomitsu et al., 2002; Shabel and Janak, 2003), and it has recently been shown that neurons in the primate amygdala increase their firing to a visual stimulus as the monkey learns to associate the stimulus with a reward, suggesting that the amygdala plays an important role in the acquisition of appetitive Pavlovian associations (Paton et al., 2006). Multiple infusions of a D3 agonist in the amygdala have been shown to enhance the acquisition of appetitive Pavlovian conditioning (Hitchcott et al., 1997a), and lesions that serve to functionally disconnect the basolateral amygdala (BLA) from the nucleus accumbens impair second-order PCA (Setlow et al., 2002). In a task where approach to the CS is the measure of conditioning, lesions of the anterior cingulate cortex and the nucleus accumbens core, as well as lesions that act to functionally disconnect these two regions, result in an impaired ability of animals to successfully discriminate between a CS+ and CS- (Parkinson et al., 2000), and infusions of D1 or NMDA receptor antagonists into the nucleus accumbens core impair learning of an appetitive Pavlovian association (Dalley et al., 2005). Some neurons within the nucleus accumbens have also been shown to fire selectively to a cue predicting a sucrose reward (Wan and Peoples, 2006). Studies examining which brain regions require the synthesis of new proteins (and, presumably, the subsequent plasticity) for the consolidation of PCA could help to further elucidate this circuit.

Post-reactivation Administration of Amphetamine and Anisomycin

In contrast to the consolidation of this memory, our results demonstrate that PCA behavior was neither positively modulated by post-reactivation AMPH injections nor negatively modulated by post-reactivation ANI injections. Studies of aversive conditioning have shown that the strength of the initial training can alter the degree to which a post-reactivation injection of ANI will affect behavior (Eisenberg et al., 2003; Suzuki et al., 2004), but in our current findings, the strength of initial training did not affect the degree of post-reactivation lability in PCA behavior. None of our post-reactivation injections affected PCA behavior, regardless of whether animals were initially trained with 2, 4, or 7 sessions.

Another interpretation of these experiments is that the treatments could be affecting extinction learning rather than reconsolidation of the original memory. During the memory reactivation sessions (as well as the test sessions), the CS+ is presented without subsequent delivery of the sucrose reward, and so these sessions also are inherently extinction sessions. In the experiments described above, a significant amount of extinction learning did take place during the memory reactivation session, as evidenced by the decrease in the difference score between the reactivation session and the first test session (see Results section). If we interpret the memory reactivation session as an extinction session, then our results show that injections of AMPH or ANI immediately after an extinction session had no effect on behavior during subsequent tests. This suggests that injections of neither AMPH nor ANI were able to modulate the consolidation of PCA extinction. In contrast to PCA, AMPH and ANI do seem to play a role in the consolidation of extinction in other types of learning. Although the role of

AMPH in extinction has not been well studied, one experiment showed that AMPH can impair extinction learning in a fear conditioning task (Kumar, 1971). In addition, injections of ANI have impaired the consolidation of extinction in multiple varieties of aversive conditioning as well as spatial memory (Berman and Dudai, 2001; Vianna et al., 2001; Lin et al., 2003; Vianna et al., 2003; Bahar et al., 2004; Suzuki et al., 2004).

We conducted two additional experiments to determine if post-reactivation injections of ANI affect PCA behavior in cases when there was little or no extinction learning during the memory reactivation session. In addition to making the results easier to interpret, this was important in light of studies showing that post-reactivation administration of protein synthesis inhibitors can have a different effect on memory depending on whether the memory reactivation produces minimal extinction learning or a large amount of extinction learning (Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Suzuki et al., 2004). However, we found that post-reactivation ANI injections still had no effect on PCA behavior when the memory reactivation session was changed in ways that reduced or eliminated extinction learning during the session. This collection of results suggests that the original PCA memory was truly unaffected by post-reactivation administration of ANI.

It remains possible that the associations formed in PCA do become labile after memory reactivation, and we simply might not have been able to uncover the lability with our current approach. For example, it is possible that the PCA memory is simply more sensitive to disruption after training than after reactivation, and administering a high dose of AMPH or ANI after reactivation might uncover post-reactivation lability. In addition, because we are ultimately interested in how cues guide reward-seeking behavior

in pathological states such as compulsive eating and addiction, our experiments used CS-induced reward-seeking behavior (i.e. approach to the sucrose port) as a measure of conditioning. However, stimuli associated with rewards can also trigger conditioned orienting responses directed toward the CS. There are distinct differences in the neural circuitry underlying conditioned orienting responses and conditioned approach responses (Gallagher et al., 1990; Setlow et al., 2002), and it is possible that our post-reactivation experimental manipulations altered the conditioned orienting responses even though there was no effect on conditioned approach responses. At the very least, however, our results show that the memory for PCA is relatively stable after memory reactivation and considerably resistant to the effects of post-reactivation injections of AMPH and ANI.

Although some types of appetitive conditioning do seem to exhibit some form of post-reactivation lability, others do not seem to be vulnerable to manipulations after memory reactivation. Post-reactivation administration of ANI does not affect behavior in an instrumental conditioning task in which rats learn to lever press for a sucrose reward (Hernandez and Kelley, 2004), and one group has shown that post-reactivation administration of ANI within the BLA does not affect morphine conditioned place preference (CPP) behavior when the memory reactivation session consists of either exposure to the CS alone or to both the CS and US (Yim et al., 2006). However, other groups have shown that post-reactivation administration of ERK kinase inhibitors or protein synthesis inhibitors (including protein synthesis inhibitors within the BLA) impair morphine CPP when the memory reactivation session consists of exposure to both the CS and US (Milekic et al., 2006; Valjent et al., 2006). When the memory reactivation session consists of exposure to the CS alone, the post-reactivation injections of AMPH

enhance morphine CPP (Blaiss and Janak, 2006), and post-reactivation inhibition of the MEK, an ERK kinase impairs cocaine CPP (Miller and Marshall, 2005). In addition, infusions of zif268 antisense oligodeoxynucleotides into the basolateral amygdala immediately before re-exposure to a cocaine-associated stimulus prevents cue-induced reinstatement of cocaine-seeking behavior and eliminates the acquired conditioned reinforcing properties of the cocaine-associated stimulus (Lee et al., 2005; Lee et al., 2006). Also, systemic post-reactivation injections of β-adrenergic antagonist propranolol impair responding in a task where rats are trained to nose poke for a sucrose reward (Diergaarde et al., 2006).

The reasons behind these conflicting findings are currently unknown. Since the concept of post-reactivation lability of memories was first introduced in the 1960s, the results from different labs have been inconsistent (Misanin et al., 1968; Dawson and McGaugh, 1969). In addition to the studies investigating appetitive conditioning (mentioned above), recent studies examining other types of memories have also found that certain post-reactivation manipulations have either no effect or only a transitory effect on behavior (Biedenkapp and Rudy, 2004; Cammarota et al., 2006; Power et al., 2006; Prado-Alcala et al., 2006). These data suggest that the post-reactivation lability seen in some memories might represent a temporary impairment in the mechanisms underlying retrieval. They also suggest that stability is the natural state of established memories, and the post-reactivation lability seen in some memories might be due to special conditions in the experimenters' behavioral paradigms. However, it is possible that seemingly subtle differences in the behavioral paradigms used by different groups result in larger differences in the neural encoding of the memories, and those differences

might affect the relative stability of the different memories. Also, while learning in some tasks can often occur in one trial, learning in other tasks (including appetitive conditioning tasks like PCA) requires multiple trials. It seems likely that this difference is somehow reflected in the neurobiology underlying these associations, and it is possible that, once learned, associations that require multi-trial learning are more stable than associations that require less training. It is also possible that there is a difference between appetitive conditioning tasks that use drugs of abuse as reinforcers and those that use natural rewards as reinforcers; however, as described above, appetitive conditioning tasks using natural rewards as reinforcers are sometimes vulnerable and sometimes resistant to post-reactivation experimental manipulations, and the same is true for tasks that use drugs of abuse as reinforcers. Future experiments may shed light on the reasons underlying the discrepancies in the literature.

In general, more study is needed to better understand the dynamic properties of the long-term memories developed in appetitive conditioning. Because cues that have previously been associated with a drug or food reward can induce relapse behavior in addicts and bouts of overeating in binge eaters (Rohsenow et al., 1994; Jansen, 1998; Sobik et al., 2005), it is tempting to think that the disruption of cue-reward associations (like those formed in the PCA task) after memory reactivation could be used as a new way to treat such disorders. However, the current findings suggest that not all associations between cues and rewards will prove to be easily disrupted after reactivation of the memory. If there is to be serious investigation into the possibility of using post-reactivation treatments for psychopathologies involving appetitive learning, then it will be important to ensure that the memory tasks used by basic scientists to develop these

treatments are accurate models for the learning that occurs in the relevant human conditions.

In summary, our results have implications for both basic science and clinical research. We have shown that AMPH enhances the consolidation of PCA, and ANI impairs it, suggesting that the consolidation of the associations learned in PCA can be modulated in a manner comparable to other types of learned associations. However, post-reactivation administration of AMPH or ANI does not affect PCA behavior. This suggests that, once learned, the PCA memory is relatively robust and stable. Therefore, it appears that the degree of post-reactivation stability differs across types of appetitive conditioning. The mechanisms underlying these differences need to be better understood if strategies based on the post-reactivation lability of memories are to be useful in the treatment of human psychopathologies that involve appetitive conditioning, such as addiction and compulsive eating disorders.

References

Abel T, Lattal KM (2001) Molecular mechanisms of memory acquisition, consolidation, and retrieval. *Current Opinion in Neurobiology*, 11:180-187.

Alberini CM (2005) Mechanisms of memory stablization: are consolidation and reconsolidation similar or distinct processes? *Trends in Neurosciences*, 28:51-56.

Bahar A, Dorfman N, Dudai Y (2004) Amygdalar circuits required for either consolidation or extinction of taste aversion memory are not required for reconsolidation. *European Journal of Neuroscience*, 19:1115-1118.

Berman DE, Dudai Y (2001) Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. *Science*, 291:2417-2419.

Biedenkapp JC, Rudy JW (2004) Context memories and reactivation: constraints on the reconsolidation hypothesis. *Behavioral Neuroscience*, 118:956-964.

Blaiss CA, Janak PH (2006) Post-training and post-reactivation administration of amphetamine enhances morphine conditioned place preference. *Behavioural Brain Research*, 171:329-337.

Brown RW, Bardo MT, Mace DD, Phillips SB, Kraemer P (2000) D-amphetamine facilitation of Morris water task performance is bloced by eticlopride and correclated with increase dopamine synthesis in the prefrontal cortex. *Behavioural Brain Research*, 114:135-143.

Cammarota M, Bevilaqua LRM, Medina JH, Izquierdo I (2006) Retrieval does not induce reconsolidation of inhibitory avoidance memory. *Learning and Memory*, 11:572-578.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. *Proc Natl Acad Sci USA*, 102:6189-6194.

Davis HP, Squire LR (1984) Protein Synthesis and Memory: A Review. *Psychological Bulletin*, 96:518-559.

Dawson RG, McGaugh JL (1969) Electroconvulsive shock effects on a reactivated memory trace: further examination. *Science,* 166:525-527.

Diergaarde L, Schoffelmeer ANM, De Vries TJ (2006) B-adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation. *Behavioural Brain Research*, 170:333-336.

Doty BA, Doty LA (1966) Facilitative effects of amphetamine on avoidance conditioning in relation to age and problem difficulty. *Psychopharmacologia*, 9:234-241.

Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol*, 55:51-86.

Dudai Y (2006) Reconsolidation: the advantage of being refocused. *Current Opinion in Neurobiology*, 16:1-5.

Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominance. *Science*, 301:1102-1104.

Epstein HT, Child FM, Kuzirian AM, Alkon DL (2003) Timew indows for effects of protein synthesis inhibitors on Pavlovian conditioning in *Hermissenda*: Behavioral aspects. *Neurobiology of learning and memory*, 79:127-131.

Fenu S, Di Chiara G (2003) Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D_1 receptors. *European Journal of Neuroscience*, 18:2025-2030.

Gallagher M, Graham PW, Holland PC (1990) The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. *J Neurosci*, 10:1906-1911.

Haycock JW, Van Buskirk R, Gold PE (1977) Effects on retention of posttraining amphetamine injections in mice: interaction with pretraining experience. *Psychopharmacology*, 54:21-24.

Hernandez PJ, Kelley AE (2004) Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. *Learning and Memory*, 11:748-754.

Hernandez PJ, Sadeghian K, Kelley AE (2002) Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. *Nature Neuroscience*, 5:1327-1331.

Hitchcott PK, Bonardi CMT, Phillips GD (1997a) Enhanced stimulus-reward learning by intra-amygdala administration of a D_3 dopamine receptor agonist. *Pyschopharmacology*, 133:240-248.

Hitchcott PK, Harmer CJ, Phillips GD (1997b) Enhanced acquistion of discriminative approach following intra-amygdala d-amphetamine. *Psychopharmacology*, 132:237-246.

Hui IR, Hui GK, Roozendaal B, McGaugh JL, Weinberger NM (2006) Posttraining handling facilitates memory for auditory-cue fear conditioning in rats. *Neurobiology of learning and memory,* 86:160-163.

Janak PH, Martinez JL, Jr. (1992) Cocaine and amphetamine facilitate retention of jump-up responding in rats. *Pharmacology, Biochemistry and Behavior,* 41:837-840.

Jansen A (1998) A learning model of binge eating: cue reactivity and cue exposure. *Behaviour Research and Therapy,* 36:257-272.

Judge ME, Quartermain D (1982) Characteristics of retrograde amnesia following reactivation of memory in mice. *Physiology and Behavior,* 28:585-590.

Krivanek JA, McGaugh JL (1969) Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. *Agents and Actions,* 1:36-42.

Kulkarni AS (1968) Facilitation of instrumental avoidance learning by amphetamine: an analysis. *Psychopharmacologia,* 13:418-425.

Kumar R (1971) Extinction of fear I: Effects of amylobarbione and dexamphetamine given separately and in combination on fear and exploratory behavior in rats. *Psychopharmacologia*, 19:163-187.

Lee EH, Ma YL (1995) Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. *Brain Research Bulletin*, 37:411-416.

Lee JLC, Milton AL, Everitt BJ (2006) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *J Neurosci,* 26:5881-5887.

Lee JLC, Di Chiano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron*, 47:795-801.

Lin C-H, Yeh S-H, Lu H-Y, Gean P-W (2003) The similarities and diversities of signal pathways leading to consolidation of conditioning and consolidation of extinction of fear memory. *Journal of Neuroscience*, 23:8310-8317.

Martinez JL, Jr., Jensen RA, Messing RB, Vasquez BJ, Soumireu-Mourat B, Geddes D, Liang KC, McGaugh JL (1980) Central and peripheral actions of amphetamine on memory storage. *Brain Research*, 182:157-166.

McGaugh JL (1966) Time-dependent processes in memory storage. *Science*, 153:1352-1258.

McGaugh JL (2000) Memory -- a century of consolidation. *Science*, 287:248-251.

McGaugh JL (2002) Memory consolidation and the amygdala: a systems perspective. *Trends in Neurosciences*, 25:456-461.

McGaugh JL, Petrinovich LF (1965) Effects of drugs on learning and memory. *International Review of Neurobiology*, 8:139-196.

Meiri N, Rosenblum K (1998) Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. *Brain Research*, 789:48-55.

Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. *Neuron*, 36:521-525.

Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. *J Neurosci*, 26:3010-3020.

Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron*, 47:873-884.

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science*, 160:554-555.

Nader K, Schafe GE, LeDoux JE (2000a) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, 406:722-726.

Nader K, Schafe GE, LeDoux JE (2000b) The labile nature of consolidation theory. *Nature Reviews Neuroscience*, 1:216-219.

Oscos A, Martinez JL, Jr., McGaugh JL (1988) Effects of post-training *d*-amphetamine on acquisition of an appetitive autoshaped lever press response in rats. *Psychopharmacologia*, 95:132-134.

Packard MG, White NM (1989) Memory facilitation produced by dopamine agonists" role of receptor subtype and mnemonic requirements. *Pharmacology, Biochemistry and Behavior*, 33:511-518.

Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behavioral Neuroscience*, 114:42-63.

Paton JJ, Belova MA, Morrison SE, Salzman CD (2006) The primate amygdala represents the positive and negative value of visual stimuli during learning. *Nature*, 439:865-870.

Pedreira ME, Maldonado H (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron*, 38:863-869.

Phillips GD, Harmer CJ, Hitchcott PK (2002) Blockade of sensitisation-induced facilitation of appetitive conditioning by post-session intra-amygdala nafadotride. *Behavioural Brain Research*, 134:249-257.

Phillips GD, Setzu E, Hitchcott PK (2003) Facilitation of Appetitive Pavlovian Conditioning by *d*-Ampetamine in the Shell, but Not the Core, of the Nucleus Accumbens. *Behavioral Neuroscience*, 117:675-684.

Power AE, Berlau DJ, McGaugh JL, Steward O (2006) Anisomycin infused into the hippocampus fails to block "reconsolidation" but impiars extinction: the role of re-exposure duration. *Learning and Memory*, 13:27-34.

Prado-Alcala RA, Diaz del Guante MA, Garin-Aguilar ME, Diaz-Trujillo A, Quirarte GL, McGaugh JL (2006) Amygdala or hippocampus inactivation after retrieval induces temporary memory deficit. *Neurobiol Learn Mem*, 86:144-149.

Rohsenow DJ, Monti PM, Rubonis AV, Sirota AD, Niaura RS, Colby SM, Wunschel SM, Abrams DB (1994) Cue reactivity as a predictor of drinking among male alcoholics. *Journal of Consulting and Clinical Psychology*, 62:620-626.

Rosenblum K, Meiri N, Dudai Y (1993) Taste memory: the role of protein synthesis in gustatory cortex. *Behavioral and Neural Biology*, 59:49-56.

Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. *Learning and Memory*, 7:73-84.

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *Journal of Neuroscience*, 20:1-5.

Setlow B, Holland PC, Gallagher M (2002) Dissconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned response. *Behavioral Neuroscience*, 116:267-275.

Shabel SJ, Janak PH (2003) Neural Correlates of Appetitive Pavlovian Conditioning in the Amygdala, Program No. 292. *2003 Abstract Viewer/Itinerary Planner*, Washington DC, Society for Neuroscience, 2003. Online.

Simon NW, Setlow B (2006) Post-training amphetamine administration enhances memory consolidation in appetitive Pavlovian conditioning: Implications for drug addiction. *Neurobiology of learning and memory*, 86:305-310.

Sobik L, Hutchison K, Craighead L (2005) Cue-elicited craving for food: a fresh approach to the study of binge eating. *Appetite*, 44:253-261.

Soetens E, D'Hooge R, Hueting JE (1993) Amphetamine enhances human-memory consolidation. *Neuroscience Letters*, 161:9-12.

Soetens E, Casaer S, D'Hooge R, Hueting JE (1995) Effect of amphetamine on long-term retention of verbal material. *Psychopharmacology*, 119:115-162.

Squire LR, Barondes SH (1974) Anisomycin, like other inhibitors of cerebral protein synthesis, impairs 'long-term' memory of a discrimination task. *Brain Research*, 66:301-308.

Squire LR, Davis HP (1975) Cerebral protein synthesis inhibition and discrimination training: effects of extent and duration of inhibition. *Behavioral Biology,* 13:49-57.

Strupp BJ, Bunsey M, Levitsky D, Kesler M (1991) Time-dependent effects of post-trial amphetamine treatment in rats: evidence for enhanced storage of representational memory. *Behavioral and Neural Biology,* 56:62-76.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci,* 24:4787-4795.

Taubenfeld SM, Milekic MH, Monti B, Alberini CM (2001) The consolidation of new but not reactivated memory requires hippocampal C/EBPβ. *Nature Neuroscience*, 4:813-818.

Toyomitsu Y, Nishijo H, Uwano T, Kuratsu J, Ono T (2002) Neuronal responses of the rat amygdala during extinction and reassociation learning in elementary and configural associative tasks. *European Journal of Neuroscience*, 15:753-768.

Valjent E, Corbille A-G, Bertran-Gonzalez J, Herve D, Girault J-A (2006) Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *PNAS,* 103:2932-2937.

Vianna MRM, Szapiro G, McGaugh JL, Medina JH, Izquierdo I (2001) Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. *Proc Natl Acad Sci USA*, 98:12251-12254.

Vianna MRM, Igaz LM, Coitinho AS, Medina JH, Izquierdo I (2003) Memory extinction requires gene expression in rat hippocampus. *Neurobiology of learning and memory*, 79:199-203.

Wan X, Peoples LL (2006) Firing patterns of accumbal neurons during a Pavlovian-conditioned approach task. *Journal of Neurophysiology*, 96:652-660.

Yim AJ, Moraes CRG, Ferreira TL, Oliveira MGM (2006) Protein synthesis inhbition in the basolateral amygdala following retrieval does not impair expression of morphine-associated conditioned place preference. *Behavioural Brain Research*, 171:162-169.

Figure Legends

Figure 1. Effect of post-training injections of amphetamine on PCA behavior

a) Experimental design

b) PCA behavior during the training sessions expressed as a mean difference score (the number of port entries during the CS+ minus the number of port entries during the CS-) ± SEM (vehicle, n=15; AMPH – imm, n=12; AMPH – 3 hrs, n=7).

c) PCA behavior during Training Session 2 expressed as a mean difference score ± SEM (vehicle, n=15; AMPH – imm, n=12; AMPH – 3 hrs, n=7). The data from the session is separated into 3 bins that each consist of 5 individual trials. *P<.05

Figure 2. Effect of delayed post-training injections of amphetamine on PCA behavior

a) Experimental design

b) PCA behavior during the training sessions expressed as a mean difference score ± SEM (vehicle, n=7; AMPH – 6 hrs, n=8).

c) PCA behavior during Training Session 2 expressed as a mean difference score ± SEM (vehicle, n=7; AMPH – 6 hrs, n=8). The data from the session is separated into 3 bins that each consist of 5 individual trials.

Figure 3. Effect of post-training injections of anisomycin on PCA behavior

a) Experimental design

b) PCA behavior during the training sessions expressed as a mean difference score ± SEM (vehicle, n=15; ANI – imm, n=7; ANI – 3 hrs, n=7). **P<.01

c) Experimental design using a lower dose of anisomycin

d) PCA behavior during the training sessions expressed as a mean difference score ± SEM (vehicle, n=8; ANI, n=8).

Figure 4. Effect of post-training injections of anisomycin on sucrose preference.

a) Experimental Design

b) Sucrose preference expressed as a mean sucrose preference ratio (the volume of sucrose consumed divided by total volume of liquid consumed) ± SEM. (vehicle, n=8; ANI, n=8)

c) PCA behavior during the training sessions expressed as a mean difference score ± SEM (vehicle, n=8; ANI, n=8). *P<.05

Figure 5. Effect of post-reactivation injections of anisomycin on PCA behavior

a) Experimental Design

b) Effect of post-reactivation injections of anisomycin when the initial training consists of 2 sessions. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=19; ANI, n=20)

c) Effect of post-reactivation injections of anisomycin when the initial training consists of 4 sessions. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=8; ANI, n=8)

d) Effect of post-reactivation injections of anisomycin when the initial training consists of 7 sessions. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=8; ANI, n=8)

Figure 6. Effect of changes to the reactivation session on the ability of post-reactivation injections of anisomycin to affect PCA behavior

a) Experimental design

b) Effect of post-reactivation injections of anisomycin when the reactivation session includes the delivery of sucrose. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=8; ANI, n=8)

c) Effect of post-reactivation injections of anisomycin when the reactivation session is shortened to include only one presentation of each CS. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=8; ANI, n=8)

Figure 7. Effect of post-reactivation injections of amphetamine on PCA behavior

a) Experimental design

b) Effect of post-reactivation injections of amphetamine when the initial training consists of 2 sessions. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=8; AMPH, n=8)

b) Effect of post-reactivation injections of amphetamine when the initial training consists of 7 sessions. PCA behavior during the reactivation and test sessions is expressed as a mean difference score ± SEM. (vehicle, n=19; AMPH, n=20)

Figure 1

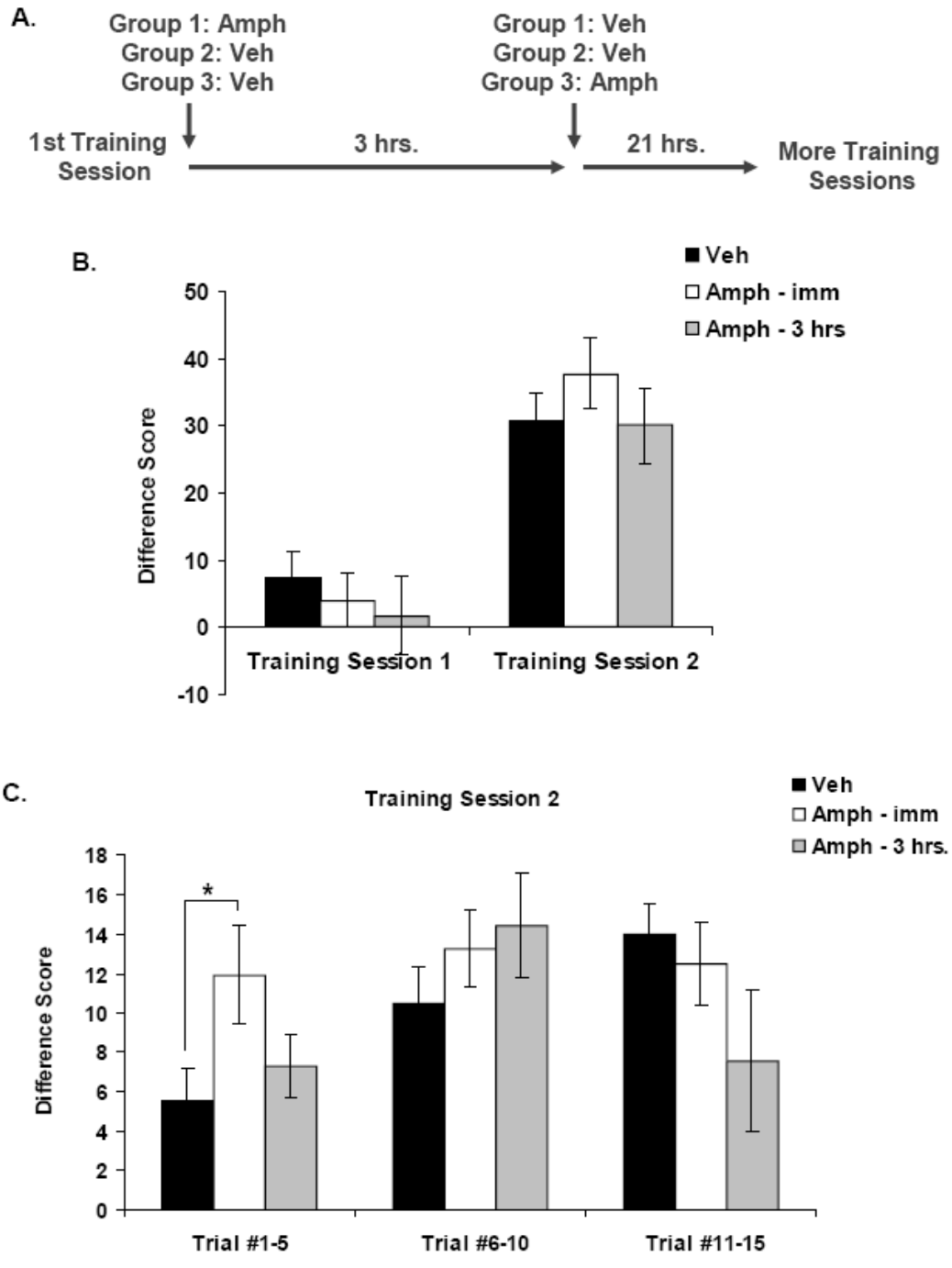

A.
Group 1: Amph
Group 2: Veh
Group 3: Veh
Group 1: Veh
Group 2: Veh
Group 3: Amph
1st Training Session
3 hrs.
21 hrs.
More Training Sessions
B.
Veh
Amph - imm
Amph - 3 hrs
Difference Score
50
40
30
20
10
0
-10
Training Session 1
Training Session 2
C.
Training Session 2
Veh
Amph - imm
Amph - 3 hrs.
Difference Score
18
16
14
12
10
8
6
4
2
0
*
Trial #1-5
Trial #6-10
Trial #11-15

Figure 2

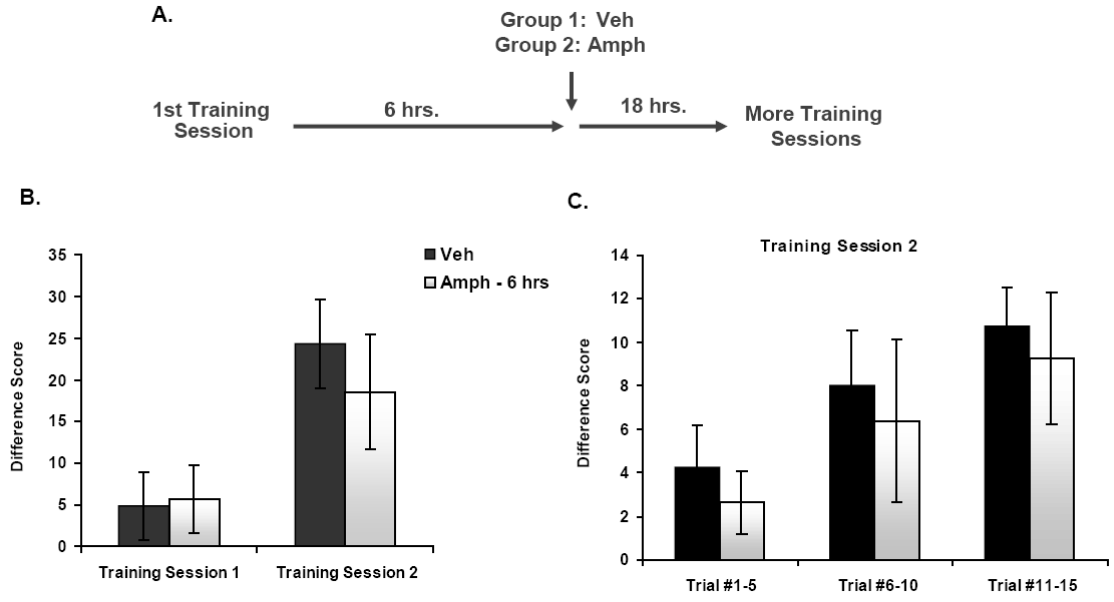

A.
Group 1: Veh
Group 2: Amph
1st Training Session
6 hrs.
18 hrs.
More Training Sessions
B.
Veh
Amph - 6 hrs
Difference Score
35
30
25
20
15
10
5
0
Training Session 1
Training Session 2
C.
Training Session 2
Difference Score
14
12
10
8
6
4
2
0
Trial #1-5
Trial #6-10
Trial #11-15

Figure 3

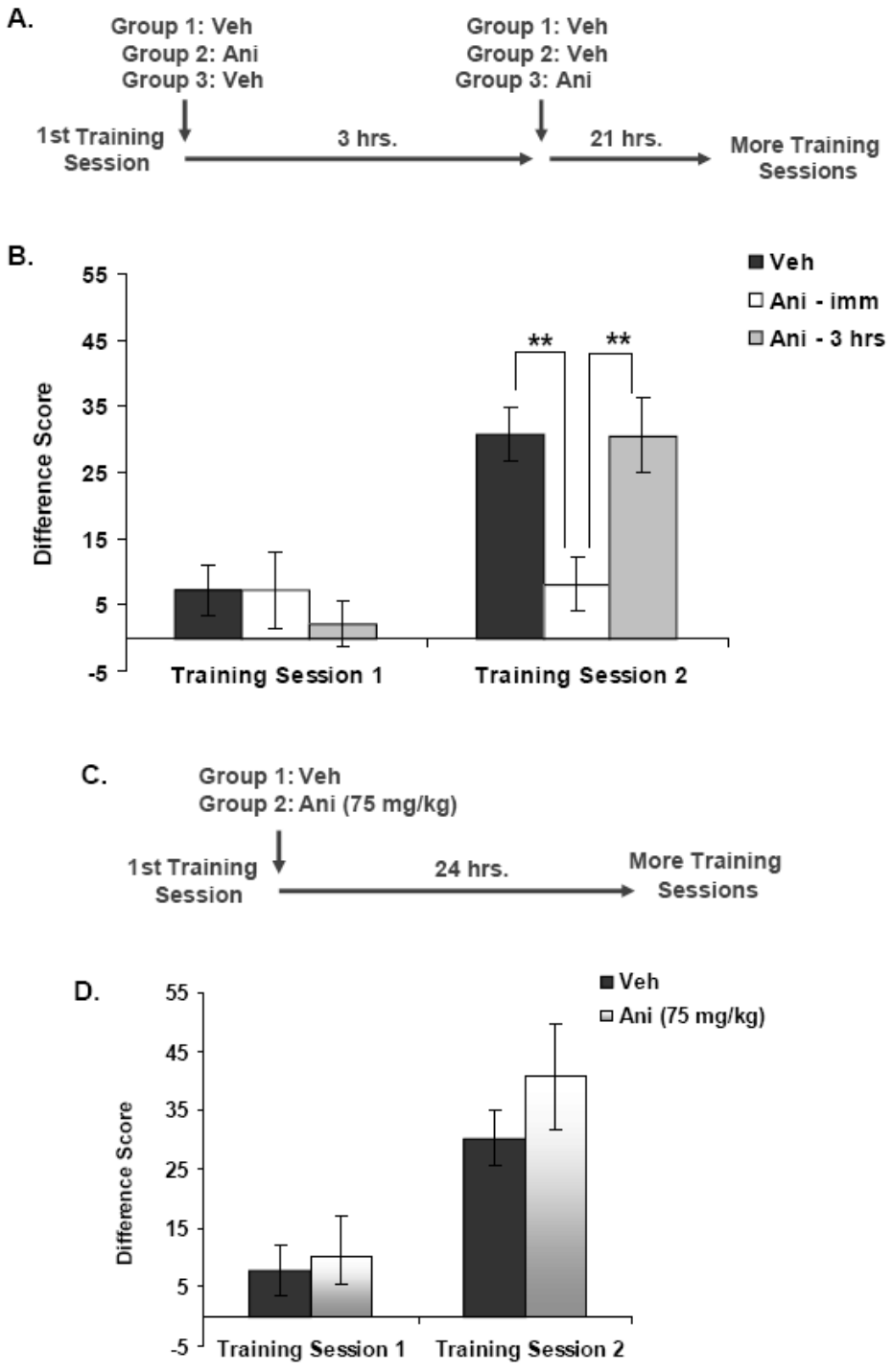

A.
Group 1: Veh
Group 2: Ani
Group 3: Veh
Group 1: Veh
Group 2: Veh
Group 3: Ani
1st Training Session
3 hrs.
21 hrs.
More Training Sessions
B.
Veh
Ani - imm
Ani - 3 hrs
Difference Score
55
45
35
25
15
5
-5
**
**
Training Session 1
Training Session 2
C.
Group 1: Veh
Group 2: Ani (75 mg/kg)
1st Training Session
24 hrs.
More Training Sessions
D.
Veh
Ani (75 mg/kg)
Difference Score
55
45
35
25
15
5
-5
Training Session 1
Training Session 2

Figure 4

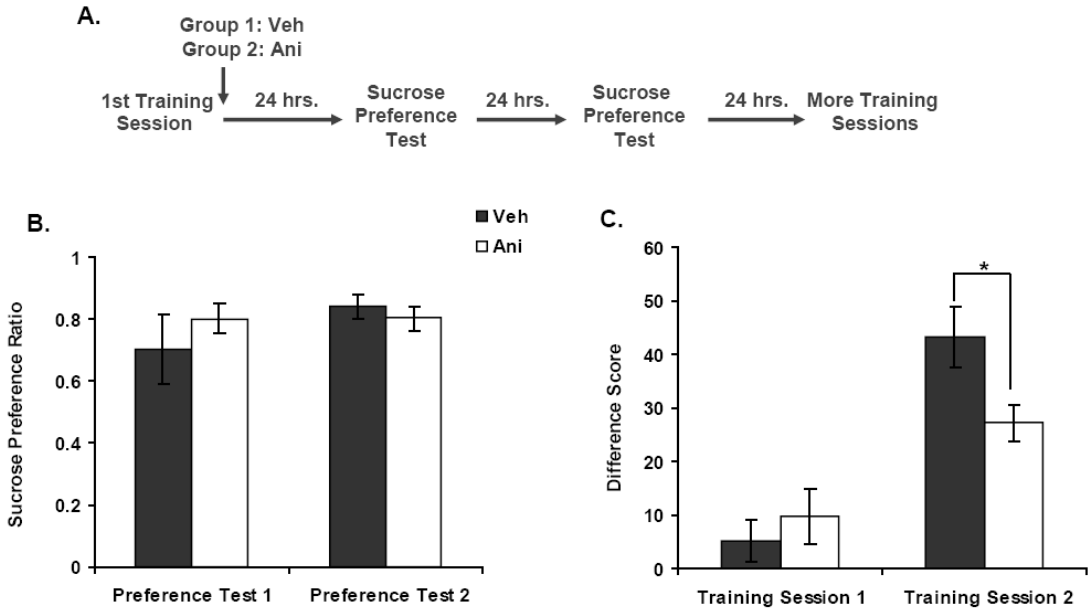

A.
Group 1: Veh
Group 2: Ani
1st Training Session
24 hrs.
Sucrose Preference Test
24 hrs.
Sucrose Preference Test
24 hrs.
More Training Sessions
B.
Veh
Ani
Sucrose Preference Ratio
1
0.8
0.6
0.4
0.2
0
Preference Test 1
Preference Test 2
C.
Difference Score
60
50
40
30
20
10
0
*
Training Session 1
Training Session 2

Figure 5

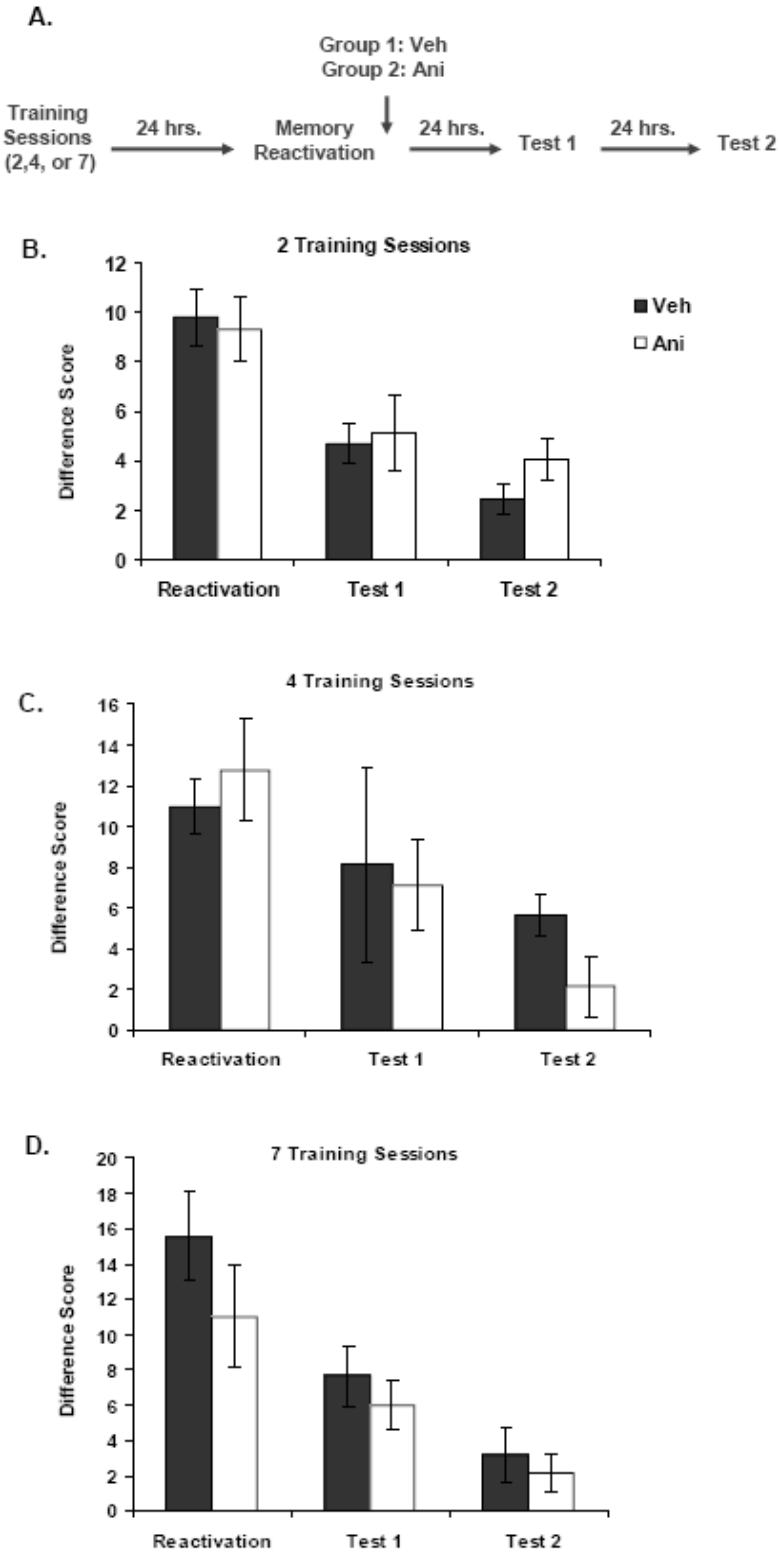

A.
Group 1: Veh
Group 2: Ani
Training Sessions (2,4, or 7)
24 hrs.
Memory Reactivation
24 hrs.
Test 1
24 hrs.
Test 2
B.
2 Training Sessions
Veh
Ani
Difference Score
Reactivation
Test 1
Test 2
C.
4 Training Sessions
Difference Score
Reactivation
Test 1
Test 2
D.
7 Training Sessions
Difference Score
Reactivation
Test 1
Test 2

Figure 6

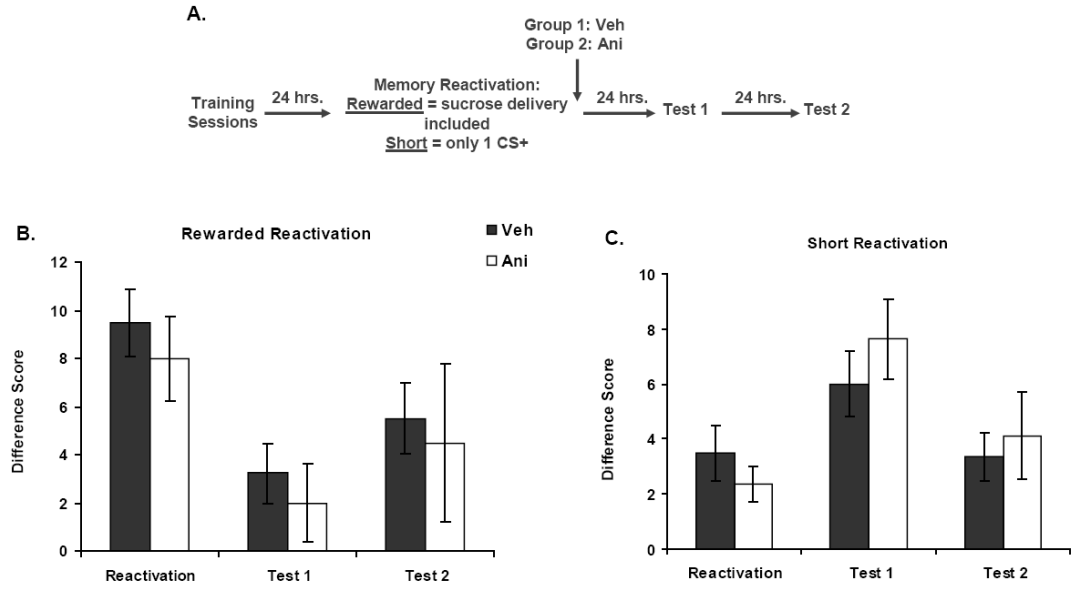
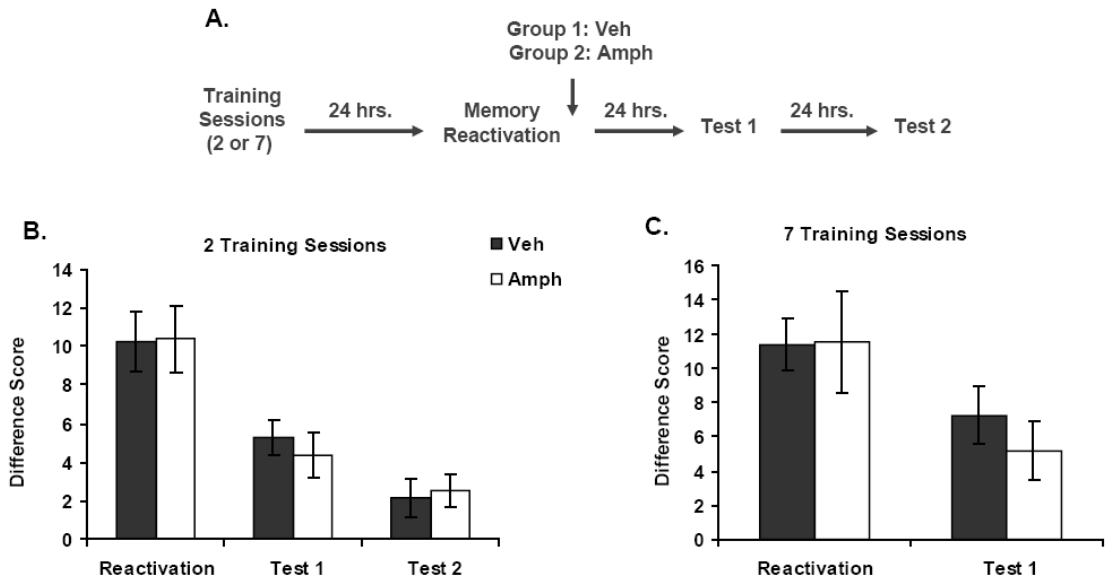

A.
Group 1: Veh
Group 2: Ani
Training Sessions
24 hrs.
Memory Reactivation:
Rewarded = sucrose delivery included
Short = only 1 CS+
24 hrs.
Test 1
24 hrs.
Test 2
B.
Rewarded Reactivation
Veh
Ani
Difference Score
Reactivation
Test 1
Test 2
C.
Short Reactivation
Difference Score
Reactivation
Test 1
Test 2

Figure 7


A.
Group 1: Veh
Group 2: Amph
Training Sessions (2 or 7)
24 hrs.
Memory Reactivation
24 hrs.
Test 1
24 hrs.
Test 2
B.
2 Training Sessions
Veh
Amph
Difference Score
Reactivation
Test 1
Test 2
C.
7 Training Sessions
Difference Score
Reactivation
Test 1

Chapter Four:

The Role of the Nucleus Accumbens in the Acquisition and Expression of Appetitive and Aversive Pavlovian Conditioning

Abstract

The nucleus accumbens (NAcc), a ventral forebrain nucleus, has been suggested to be involved in both appetitive and aversive Pavlovian conditioning. However, the role of the NAcc and its subnuclei, the core and shell, in these types of associative learning remains unclear. To further elucidate the role of the NAcc core and shell in appetitive and aversive Pavlovian conditioning, we examined the effect of reversible inactivation of these brain regions on the acquisition and expression of Pavlovian conditioned approach (PCA), a form of appetitive Pavlovian conditioning, and cued fear conditioning, a form of aversive Pavlovian conditioning. We also examined whether protein synthesis is necessary in the NAcc core and shell for the acquisition of these behaviors. Neither post-training reversible inactivation nor inhibition of protein synthesis in either the core or shell had any effect on the acquisition of PCA behavior; however reversible inactivation of both the NAcc core and shell impaired the ability of animals to discriminate between the CS+ and CS- during PCA test sessions. In contrast, both post-training reversible inactivation and post-training inhibition of protein synthesis in the NAcc core (but not the

shell) impaired the acquisition of cued fear conditioning, suggesting that protein synthesis-dependent plasticity is required in the NAcc core for the acquisition of this task. However reversible inactivation of the NAcc core and shell had no effect on the expression of cued fear conditioning. These data suggest that the NAcc is differentially involved in appetitive and aversive Pavlovian conditioning.

Introduction

Historically, the nucleus accumbens (NAcc) has been considered a site of "limbic-motor integration" that is important for the ability of motivationally significant stimuli to guide goal-directed behavior (Mogenson et al., 1980; Mogenson, 1987; Cardinal et al., 2002a). The NAcc has been shown to be involved in a variety of appetitive behaviors (Everitt et al., 1999; Cardinal et al., 2001; Corbit et al., 2001; Hall et al., 2001; Hernandez et al., 2002; Kelley, 2004; Balleine, 2005) as well as spatial and declarative learning (Setlow, 1997; Ferretti et al., 2005). However, the functional role of the NAcc and its subnuclei, the NAcc core and shell, in Pavlovian conditioning remains unclear.

Excitotoxic lesions of the NAcc core have been shown to impair both the acquisition and performance of an autoshaping task in which rats are trained to respond to a conditioned stimulus (CS) by approaching the stimulus (Parkinson et al., 2000; Cardinal et al., 2002b), and D1/D2, NMDA, and AMPA receptors within the core have been shown to be involved in this behavior (Di Chiano et al., 2001; Dalley et al., 2005). In a Pavlovian conditioned approach (PCA) task in which rats are trained to respond to a CS by approaching a reward port, excitotoxic, but not electrolytic, lesions of the NAcc core impaired behavior (Parkinson et al., 1999; Cassaday et al., 2005). In the NAcc shell,

infusions of *d*-amphetamine enhanced the acquisition of PCA (Phillips et al., 2003), but neither excitotoxic nor electrolytic lesions affected this behavior (Parkinson et al., 1999; Cassaday et al., 2005). Although these data suggest that the NAcc core and shell are both involved in appetitive Pavlovian conditioning, their exact role remains unclear, and it is not known whether plasticity underlying appetitive Pavlovian associations occurs in these regions.

Recent studies have suggested that the NAcc is involved also in behaviors where cues are associated with aversive outcomes. Excitotoxic lesions of the full NAcc impaired the acquisition of an aversive odor discrimination task (Schoenbaum and Setlow, 2003), and electrolytic, but not excitotoxic, lesions of the NAcc core impaired cued fear conditioning (Levita et al., 2002; Cassaday et al., 2005). Also, while infusions of the local anesthetic bupivicaine into the NAcc had no effect on the acquisition of cued fear conditioning, infusions of tetrodotoxin into the NAcc core impaired the acquisition and expression of fear potentiated startle (Haralambous and Westbrook, 1999; Schoenbaum and Setlow, 2003). The inconsistencies across these studies highlight how little is known about the role of the NAcc in aversive Pavlovian conditioning.

To further elucidate the role of the NAcc core and shell in appetitive and aversive Pavlovian conditioning, we have examined the effect of reversible inactivation of these brain regions on the acquisition and expression of Pavlovian conditioned approach (PCA), a form of appetitive Pavlovian conditioning, and cued fear conditioning, a form of aversive Pavlovian conditioning. Additionally, we have used the protein synthesis inhibitor anisomycin to investigate whether protein synthesis-dependent plasticity is necessary within these brain regions for the acquisition of these Pavlovian associations.

Materials and Methods

Subjects

Male Long-Evans rats (Harlan, Indianapolis, IN) weighing between 250-280g were individually housed on ventilated racks in polycarbonate cages, and subjects were kept on a 12-h light:12-h dark cycle (lights on at 7 a.m.). The day before the start of behavioral training in the Pavlovian Conditioned Approach (PCA) task, rats were started on a schedule of water restriction; they were allowed free access to water for two hours per day, immediately after behavioral sessions. After the last PCA training session, rats once again received water ad libitum. All rats received food ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center at the University of California, San Francisco, and are in accordance with "PHS Policy on Humane Care and Use of Laboratory Animals," Office of Laboratory Animal Welfare, National Institutes of Health, USA, revised 2002.

Surgical Procedures

Rats were anesthetized with isoflurane (Baxter, Deerfield, IL) at an initial dose of 5% w/v for induction; the dose was gradually decreased to approximately 2% w/v for maintenance. Guide cannulae (26 gauge, Plastics One, Roanoke, VA) were implanted bilaterally into the nucleus accumbens (NAcc) core or shell using standard stereotaxic procedures. Stereotaxic coordinates for the NAcc core were 1.5 mm anterior to bregma, 1.5 mm from the midline, and 5.4 mm ventral (measured from dura); stereotaxic coordinates for the NAcc shell were 1.4 mm anterior to bregma, .75 mm from the

midline, and 5.0 mm ventral (measured from dura). As an analgesic, all rats were given ad lib access to acetaminophen (.4 mg/mL, oral, McNeil Consumer and Specialty Pharmaceuticals, Fort Washington, PA) for several days following surgery, and they were allowed at least 5 days to recover before the start of handling and behavioral procedures.

Drugs and Infusion Procedures

Internal cannulae (33 gauge, Plastics One, Roanoke, VA) were attached to 25 μL syringes (Hamilton, Reno, NV) through polyethylene tubing filled with distilled water. Drugs were separated from the distilled water by a small air bubble, and infusions were controlled by a syringe pump (Harvard Apparatus, Holliston, MA). For infusions into the NAcc core, the internal cannulae extended 1.0 mm past the end of the guide cannulae, and for infusions into the NAcc shell, the internal cannulae extended 2.0 mm past the end of the guide cannulae. Rats were held gently by the experimenter during the infusion.

Anisomycin (Sigma, St. Louis, MO) was dissolved in 1N HCl, adjusted to a pH of 7.2, and diluted in phosphate-buffered saline. Anisomycin was infused at a dose of 62.5 μg per .5 μL per hemisphere. This dose was chosen because it has been shown to impair the consolidation of instrumental conditioning when infused into the NAcc core (Hernandez et al., 2002), and a similar dose has been shown to cause >90% inhibition of protein synthesis in the hippocampus within 10 min of infusion into the lateral ventricle (Meiri and Rosenblum, 1998)and >90% inhibition of protein synthesis for >2 hr in the cortex when infused locally(Rosenblum et al., 1993). The drug was infused at a rate of .25 μL/min, and the internal cannulae were left in place for 1 additional minute to allow for diffusion.

The GABA agonists muscimol and baclofen (Sigma, St. Louis, MO) were both dissolved in phosphate-buffered saline at concentrations of .1 mM for muscimol and 1.0 mM for baclofen, and the muscimol/baclofen combination was infused at a total volume of .3 μL per hemisphere. Infusions of this dose into the nucleus accumbens have previously been shown to impair responding in operant appetitive tasks (N. Chaudhri and P.H. Janak, unpublished observations; (McFarland and Kalivas, 2001). The muscimol/baclofen combination (M/B) was infused at a rate of .3 μL/min, and the internal cannulae were left in place for 2 additional minutes to allow for diffusion.

Histology

Rats were deeply anesthetized with pentobarbital (190 mg/kg, IP, Virbac AH, Fort Worth, TX) and transcardially perfused with phosphate-buffered saline followed by 10% formalin (Fisher Scientific, Fair Lawn, NJ). Brains were extracted and sliced on a microtome at a thickness of 50 μm. Slices were stained with thionin and examined under a light microscope for accurate cannula placements. Cannula placement was determined through comparison with an atlas of the rat brain (Paxinos and Watson, 1998), and rats with inaccurate cannula placements were discarded from the study.

Behavioral Procedures

General Behavioral Apparatus

All behavioral experiments were conducted in conditioning chambers (MedAssociates, Georgia, VT) that were housed in sound-attenuating chambers. Syringe pumps delivered sucrose into a rectangular recess (sucrose port) located on the right side of the chamber,

and photobeams detected a rat's entrance into the sucrose port. Footshocks were delivered through a grid floor that was connected to a shock generator. A stimulus light and a houselight were located in different places on the wall of the chamber, and a 2.9 kHz tone or white noise could be played from speakers on the wall of the chamber. A computer with MedAssociates software controlled all the equipment in the conditioning chambers, and entries into the sucrose port were recorded by the software.

Pavlovian Conditioned Approach (PCA) Procedures

In order to teach rats to consume sucrose from the sucrose port, rats were first given a single habituation session that lasted approximately 1 hr. After a 5 min habituation period, 30 deliveries of a 10% sucrose solution (w/v, .2 mL) were given on a VI-120 s schedule, and no conditioned stimuli were presented. All subsequent PCA sessions were training sessions that lasted 90 min, and training sessions were separated by 24 h. The training sessions began with a 5 min habituation period, and then presentations of a CS+ and a CS- (15 presentations of each) were given in random order on a VI-150 s schedule. The stimuli used were a 2.9 kHz tone and a lighting change (houselight off, stimulus light on); the choice of stimulus to be used as the CS+ was counterbalanced across subjects. Stimulus presentations (both CS+ and CS-) lasted for 10 s, and a 10% sucrose solution (w/v, .2 mL) was delivered immediately after the offset of the CS+.

The number and duration of sucrose port entries during were recorded by computer. To use normalized measures of conditioning that compared behavior during the CS+ and CS-, we subtracted the number of port entries during the CS- from the number of port entries during the CS+, and we termed this measure of behavior the difference score. To examine whether the rats exhibited a basic motivation to consume

the sucrose solution, we also determined whether the rats responded to the US delivery on each trial. On any given trial, a rat was defined as making a US response if the rat entered the sucrose port within 30 s of the onset of the syringe pump that delivered the reward.

Cued Fear Conditioning Procedures

Subjects were given at least 3 days to rest in their home cages between the end of the PCA behavioral sessions and the beginning of fear conditioning, and all of the fear conditioning sessions took place in separate conditioning chambers from the PCA sessions. Experimental groups for the fear conditioning experiments were all counterbalanced for previous drug experience and for the stimulus chosen as the CS+ during the PCA experiments.

During the fear conditioning training session, there was a 5 min habituation period followed by a single 30 s presentation of a compound CS that consisted of white noise and a lighting change (houselight on, stimulus light off). The CS co-terminated with a 2 mA, 1 s footshock. Twenty-four hours later, subjects were given a test session that was conducted in a different context from training (different conditioning chamber, solid floor instead of a grid floor, and the addition of a strawberry air freshener). During the test session, there was a 5 min habituation period followed by 5 presentations of the compound CS. Each CS presentation was 30 s long, and presentations were separated by 30 s.

Fear conditioning sessions were recorded onto videotape by a video camera located at the top of the conditioning chambers, and the total time spent freezing during the CS presentations was scored visually by a blind observer. Behavior was considered

freezing if the rat had all four legs on the floor and there was no visible movement. Locomotor activity was scored during the last 3 min of the habituation period. The floor of the conditioning chamber was divided into quadrants, and a blind observer visually tallied the number of times the rat crossed the lines separating the quadrants. A rat was defined as crossing a line when an imaginary pixel in the center of the rat's shoulder blades crossed the line.

Statistical Analysis

The data were initially analyzed using 1-way or 2-way repeated measures ANOVAs, and depending on the experiment, Treatment or Treatment Order was used as a between-subjects factor, and Session, CS Number, or Treatment was used as a within-subjects factor. Significant main effects and/or interactions were further analyzed through planned comparisons, and all statistical analyses were conducted using SigmaStat software (SPSS, Inc, Chicago, IL).

Results

Acquisition of Pavlovian Conditioned Approach

To examine whether protein synthesis is necessary in either the nucleus accumbens (NAcc) core or shell for the acquisition of Pavlovian conditioned approach (PCA), we tested whether immediate post-training infusions of either vehicle or the protein synthesis inhibitor anisomycin (ANI) would impair learning of PCA. After one habituation session (see methods), rats were given a series of PCA training sessions, each separated by 24 h. Rats were given infusions of either vehicle or ANI into the NAcc core or shell

immediately after each of the first three training sessions (see Figure 1a for experimental design). Rats were given post-training infusions in order to examine the effect of the drugs on between-session learning while avoiding any possible confounds due to an effect of the drug on performance. Rats with inaccurate cannula placements were discarded from the study, and schematic representations of the cannula placements are shown in Supplementary Figure 1a (for the NAcc core) and Supplementary Figure 1b (for the NAcc shell).

Infusions of ANI into the NAcc core had no effect on PCA behavior (see Figure 1b). A 2-way repeated measures ANOVA analyzing the difference scores across training sessions found a significant main effect of Session [$F(3,21)=52.810$; $P<.001$], but there was neither a significant main effect of Treatment [$F(1,21)=.001$; $P=.981$] nor a significant interaction between the Treatment and Session [$F(3,21)=1.248$; $P=.300$]. Likewise, post-training infusions of ANI into the NAcc shell also had no effect on PCA behavior. Figure 1c shows the difference score during the PCA training sessions for rats that received infusions into the NAcc shell, and while a 2-way repeated measures ANOVA did find a significant main effect of Session [$F(3,10)=30.227$, $P<.001$], there was neither a significant main effect of Treatment [$F(1,10)=2.190$, $P=.17$] nor a significant interaction between the Treatment and Session [$F(3,10)=.325$; $P=.807$]. These data suggest that protein synthesis within the NAcc is not required for acquisition of the association underlying PCA behavior.

Since protein synthesis dependent plasticity does not appear to be necessary for the acquisition of PCA, we hypothesized that general neural activity in the nucleus might be necessary during acquisition in order to modulate plasticity occurring in other brain

regions. Therefore, we next examined whether reversible inactivation of the NAcc core or shell would impair the acquisition of PCA. After one habituation session, rats were given a series of PCA training sessions, each separated by 24 h. A cocktail of muscimol, a $GABA_A$ agonist, and baclofen, a $GABA_B$ agonist, was used to reversibly inactivate the NAcc core or shell, and infusions of either vehicle or the muscimol/baclofen cocktail (M/B) were infused into the core or the shell immediately after each of the first three PCA training sessions (see Figure 2a for the experimental design). Rats with inaccurate cannula placements were discarded from the study, and schematic representations of the cannula placements are shown in Supplementary Figure 2a (for the NAcc core) and Supplementary Figure 2b (for the NAcc shell).

Infusions of M/B into the NAcc core had no effect on the mean difference score during PCA training sessions (see Figure 2b). While a 2-way repeated measures ANOVA did find a significant main effect of Session [$F(3,11)=2.005$, $P<.001$], there was neither a significant main effect of Treatment nor a significant interaction between factors [Treatment: $F(1,11)=.196$, $P=.667$; Interaction: $F(3,11)=.069$, $P=.976$]. Similarly, infusions of M/B into the NAcc shell also had no effect on PCA behavior. The mean difference scores during PCA training sessions for rats that received infusions into the NAcc shell are shown in Figure 2c. A 2-way repeated measures ANOVA found no significant main effect of Treatment, although there was a significant main effect of Session [Treatment: $F(1,13)=.103$, $P=.753$; Session: $F(3,13)=12.624$, $P<.001$; Interaction: $F(3,13)=.159$, $P=.923$]. Post-training infusions of ANI and post-training infusions of M/B did not affect the differences scores of rats during PCA training sessions, regardless of whether these drugs were administered into the NAcc core or shell. These data

suggest that neither new protein synthesis nor general neural activity is required in the NAcc for successful acquisition of the association underlying PCA.

Expression of Pavlovian Conditioned Approach

To determine whether neural activity in the NAcc is necessary for the expression of PCA, we investigated whether reversible inactivation of the NAcc core and shell immediately before testing would impair PCA behavior. After a habituation session, rats were given a series of PCA training sessions, each separated by 24 h. The fifth and sixth training sessions served as test sessions, and infusions of either vehicle or M/B were administered approximately 5 min before the each of these sessions. This experiment used a within subjects design, and the subjects were counterbalanced for treatment order. Therefore, half of the rats received an infusion of vehicle before the first test session and an infusion of M/B before the second test session; the conditions were reversed for the other half of the rats (see Figure 3a for experimental design). Supplementary Figure 3a shows the cannula placements for the subjects that received infusions into the NAcc core, and Supplementary Figure 3b shows the cannula placements for the subjects that received infusions into the NAcc shell.

Figure 3b shows the difference scores during all PCA sessions for rats that received infusions in the NAcc core. There was a significant increase in the mean difference score across the four training sessions [1-way repeated measures ANOVA; $F(3,14)=31.172$, $P<.001$], suggesting that all rats successfully learned the task before experiencing the test sessions. During the test sessions, rats exhibited an impaired ability to discriminate between the CS+ and CS- when M/B was infused into the NAcc core

before the test session. A 2-way repeated measures ANOVA analyzing the difference scores during the test sessions found a significant main effect of Treatment [F(1,13)=14.088, P<.002], and there was neither a significant main effect of Treatment Order [F(1,13)=1.114, P=.310] nor a significant interaction between the two factors [F(1,13)=1.437, P=.252]. Port entry behavior from one representative rat (see Figure 3d) suggests that the decrease in difference scores seen after intra-NAcc core M/B administration was due to a markedly impaired conditioned response to the CS+. Data from the group as a whole supports this view; when rats are given M/B infusions, they make fewer port entries during the CS+ compared to vehicle infusions [Data not shown; 2-way repeated measures ANOVA; Treatment: F(1,13)=5.825, P<.031; Order: F(1,13)=1.861, P=.196; Interaction: F(1,13)=1.491, P=.244], but the number of port entries that the rats make during the 10 sec preceding the onset of the CS+ remains unchanged after M/B infusions [Data not shown; 2-way repeated measures ANOVA; Treatment: F(1,13)=.038, P=.849; Order: F(1,13)=3.39, P=.091; Interaction: F(1,13)=1.072, P=.319].

Instead of resulting from a specific effect of the drugs on the expression of the PCA association, it is possible that the impaired behavior resulting from pre-test M/B infusions could be explained by an impairing effect of the drugs on general activity or motivation to consume the US. We therefore examined two additional measures of behavior during the test sessions to determine if that was the case. In order to assess the rats' general level of activity during the test sessions, we determined the total amount of port entries made throughout the 90 min sessions. As shown in Figure 3f**,** the total number of port entries was unchanged after the infusion of M/B when compared to the

total number of port entries after the infusion of vehicle [1-way repeated measures ANOVA; $F(1,14)=.189$, $P=.671$], suggesting that the impairment in the PCA task seen after the infusion of M/B was not due to an impairment in the general level of activity. In order to assess the rats' general level of motivation to consume the US, we determined the total number of trials during which the rats made a response to consume the US (see Materials and Methods). As shown in Figure 3g, infusions of M/B into the NAcc core did not affect the number of trials during which the rats made responses to consume the US [1-way repeated measures ANOVA; $F(1,14)=2.168$; $P=.163$], suggesting that the impairment in the PCA task seen after the infusion of M/B was not due to a general impairment in the rats' motivation to consume the US. This combination of data suggests that pre-test infusions of M/B in the NAcc core specifically impaired the expression of the PCA task.

Figure 3c shows the difference scores during all PCA sessions for rats that received infusions into the NAcc shell. A 1-way repeated measures ANOVA analyzing the difference scores of all rats across the four training sessions found a significant increase in the mean difference score [$F(3,7)=17.156$, $P<.001$], suggesting that the rats had successfully learned the PCA task before the first test session. A 2-way repeated measures ANOVA analyzing the difference scores during the test sessions found a significant main effect of Treatment [$F(1,6)=30.427$, $P<.001$], suggesting that, as when it is infused into the NAcc core, infusion of M/B into the NAcc shell resulted in impaired behavior in the PCA task. In addition, this analysis also showed that the impairing effect of M/B on PCA behavior could not be explained by the order of infusions [2-way repeated measures ANOVA; Treatment Order: $F(1,6)=.201$, $P=.669$; Interaction:

F(1,6)=.647, P=.452]. Although infusions of M/B into the NAcc shell and core both resulted in impaired difference scores during the test sessions, the more specific behavioral impairments seen after infusions in the shell (see Figure 3e) differed from those seen after infusions in the core (see Figure 3d). Like rats that received M/B infusions in the core, rats that received M/B infusions in the shell also made fewer port entries during the CS+ [Data not shown; 2-way repeated measures ANOVA; Treatment: F(1,6)=16.197, P<.007; Order: F(1,6)=.301, P=.603; Interaction: F(1,6)=.101, P=.761], but in addition, these rats exhibited a greater number of port entries during the 10 sec preceding the onset of the CS+ [Data not shown; 2-way repeated measures ANOVA; Treatment: F(1,6)=7.269, P<.036; Order: F(1,6)=.050, P=.831; Interaction: F(1,6)=1.046, P=.346]. Therefore, it is possible that M/B infusions in the shell impaired the normal expression of PCA by affecting the rats' ability to inhibit unnecessary behaviors.

For this experiment, we also examined additional measures of behavior to determine if the impairing effect of M/B on PCA behavior could be explained by an impairing effect of the drugs on the rats' general activity level or motivation to consume the US. As shown in Figure 3f, rats exhibited a greater number of port entries during the test session after infusion of M/B in the NAcc shell when compared to the test session after infusion of vehicle [1-way repeated measures ANOVA; F(1,7)=12.609, P<.009]. While infusions of M/B in the NAcc shell did result in an increased general activity level, this is the opposite of the result that would be expected if the impairing effect of M/B on difference scores was due to a non-specific impairing effect on general activity level. However, this does support the view that rats' behavior after intra-NAcc shell M/B infusions becomes less specific and efficient. As shown in Figure 3g, infusions of M/B

into the NAcc shell did not affect the number of trials during which the rats made responses to consume the US [1-way repeated measures ANOVA; $F(1,7)=4.200$; $P=.08$], suggesting that the impairment in the PCA task seen after the infusion of M/B was not due to a general impairment in the rats' motivation to consume the US. This combination of data suggests that pre-test infusions of M/B in the NAcc shell specifically impaired normal expression of the PCA task, but the specific behavioral impairments seen after infusions in the NAcc shell were slightly different that the impairments seen after infusions in the NAcc core.

Acquisition of Cued Fear Conditioning

To examine whether protein synthesis is necessary in the NAcc core or shell for the acquisition of cued fear conditioning, we tested whether immediate post-training infusions of either vehicle or the protein synthesis inhibitor anisomycin (ANI) would impair learning of cued fear conditioning. Rats were given a fear conditioning training session, and either vehicle or ANI was locally administered immediately after the training session. Supplementary Figure 4a shows the cannula placements for subjects that received infusions into the NAcc core, and Supplementary Figure 4b shows the cannula placements for subjects that received infusions into the NAcc shell. Freezing behavior was then assayed during a test session 24 h after the training session (see Figure 4a for experimental design).

Rats that received post-training infusions of ANI into the NAcc core exhibited significantly reduced freezing during the test session compared to the vehicle group [see Figure 4b; 2-way repeated measures ANOVA; Treatment: $F(1,13)=8.103$, $P<.014$; CS

Number: F(4,13)=.374, P=.826; Interaction: F(4,13)=1.285, P=.288]. Further analysis showed that the percent of time spent freezing during the test session was significantly lower in the ANI group than the vehicle group during CS presentations two through five [Student-Newman-Keuls planned pairwise comparisons; CS Number 2: P<.046; CS Number 3: P<.009; CS Number 4: P<.011; CS Number 5: P<.006]. It was still possible the decrease in freezing during the test session could be explained by a general enhancing effect of ANI on locomotor activity. However, there was no difference between the vehicle and ANI groups in the number of line crossings during the habituation period of the test session [see Figure 4c; 1-way ANOVA, Treatment: F(1,21)=.002, P=.964]. In contrast to the effect of intra-NAcc core infusions of ANI, post-training infusions of ANI into the NAcc shell had no effect on freezing during the test session (see Figure 4d). A 2-way repeated measures ANOVA analyzing the percent of time spent freezing across all 5 CS presentations found no significant effects [Treatment: F(1,16)=1.219, P=.286; CS Number: F(4,16)=2.161, P=.083; Interaction: F(4,16)=1.057, P=.385]. This combination of data suggests that protein synthesis is required within the NAcc core, but not the NAcc shell, for acquisition of cued fear conditioning.

We next investigated whether reversible inactivation of the NAcc core or shell would impair the acquisition of cued fear conditioning. Similar to the previous experiment, rats were given a fear conditioning training session, and either vehicle or M/B was locally administered immediately after the training session. Supplementary Figure 5a shows the cannula placements for subjects that received infusions into the NAcc core, and Supplementary Figure 5b shows the cannula placements for subjects that

received infusions into the NAcc shell. Freezing behavior was then assayed during a test session 24 h after the training session (see Figure 5a for experimental design).

Figure 5b shows the percent of time the rats spent freezing during the CS presentations in the test session. Although a 2-way repeated measures ANOVA analyzing the percent of time spent freezing across all CS presentations did not find a significant main effect of Treatment, there was a significant interaction between Treatment and CS Number [Treatment: $F(1,20)=3.352$, $P=.082$; CS Number: $F(4,20)=2.275$, $P=.068$; Interaction: $F(4,20)=2.695$, $P<.037$]. Further analysis found that the time spent freezing in the M/B group was significantly less than the vehicle group during CS presentations three and four [Student-Newman-Keuls planned pairwise comparisons; CS Number 3: $P<.045$; CS Number 4: $P<.016$]. As in the experiment that examined the effects of post-training intra-NAcc core infusions of ANI, it was still possible the decrease in freezing during the test session could be explained by a general enhancing effect of M/B on locomotor activity, and it was especially important to investigate this possibility in light of the fact that manipulations in the NAcc have been shown to affect general locomotor activity (Pijnenburg et al., 1973; Jackson et al., 1975; Kelly et al., 1975; Kelly and Iversen, 1976; Zahm, 2000). However, there was no difference between the vehicle and M/B groups in the number of line crossings during the habituation period of the test session [see Figure 5c; 1-way ANOVA, Treatment: $F(1,14)=3.708$, $P=.706$]. In contrast to the effect of intra-NAcc core infusions of M/B, post-training infusions of M/B into the NAcc shell did not affect freezing behavior during the test session [see Figure 5d; 2-way repeated measures ANOVA; Treatment: $F(1,13)=.087$, $P=.773$; CS Number: $F(4,13)=2.261$, $P<.045$; Interaction: $F(4,13)=.886$,

P=.479], suggesting that general neural activity in required in the NAcc core, but not the NAcc shell, for the acquisition of cued fear conditioning.

Expression of Cued Fear Conditioning

To examine whether reversible inactivation of the NAcc core or shell affects the expression of cued fear conditioning, rats were first given a fear conditioning training session. Twenty-four hours after the training session, rats were given a test session, and either M/B or vehicle was infused into the NAcc core or shell approximately 5 min before the start of the test session (see Figure 6a for the experimental design). Supplementary Figure 6a shows the cannula placements for subjects that received infusions into the NAcc core, and Supplementary Figure 6b shows the cannula placements for subjects that received infusions into the NAcc shell.

Figure 6b shows the percent of time spent freezing during the test session for the rats that received infusions into the NAcc core. A 2-way repeated measures ANOVA found no significant main effect of Treatment [F(1,13)=.436; P=.520] or CS Number [F(4,13)=1.196, P=.324]. However, because there was a significant Interaction between Treatment and CS Number {F(4,13)=2.810; P<.035], we conducted pairwise comparisons to determine whether there was any significant difference between veh and M/B treated rats during any of the individual CS presentations. Pairwise comparisons found no significant difference of Treatment for any of the 5 CS presentations [Student-Newman-Keuls pairwise comparison; CS Number 1: P=.61, CS Number 2: P=.72, CS Number 3: P=.10, CS Number 4: P=.22, CS Number 5: P=.48], suggesting that pre-test infusions of M/B into the NAcc core had no effect on freezing. Pre-test infusions of M/B

into the NAcc shell also had no effect on freezing behavior during the test session [see Figure 6c; 2-way repeated measures ANOVA; Treatment: $F(1,11)=.741$, $P=.408$; CS Number: $F(4,11)=1.997$, $P=.112$; Interaction: $F(4,11)=.535$, $P=.711$]. These data suggest that general neural activity is not necessary in the NAcc core or shell for the expression of cued fear conditioning.

Discussion

In summary, we have shown that reversible inactivation of the NAcc core and shell has no effect on the acquisition of Pavlovian conditioned approach (PCA), and protein synthesis within the NAcc core and shell is not required for the acquisition of the PCA association (see Figure 7 for a summary of results). In contrast, reversible inactivation of both the NAcc core and shell impairs the expression of PCA behavior, although reversible inactivation of these two subnuclei did result in different specific impairments in behavior. These data suggest that the NAcc is minimally involved in the acquisition of the PCA association and not the site of any protein synthesis-dependent plasticity underlying the association. In contrast, neural activity in the NAcc core and shell appears to be necessary for the normal expression of PCA behavior, but these subnuclei appear to play different roles in this behavior.

We have also shown that reversible inactivation of NAcc core, but not the NAcc shell, impairs the acquisition of cued fear conditioning. In addition, protein synthesis is required in the NAcc core, but not the NAcc shell, for the acquisition of cued fear conditioning. In contrast, reversible inactivation of the NAcc core and shell has no effect on the expression of cued fear conditioning. These data suggest that although the NAcc

as a whole does not appear to be involved in the normal expression of cued fear conditioning, the NAcc core (but not the shell) is integrally involved in the acquisition of this aversive association.

The role of the nucleus accumbens in Pavlovian conditioned approach

Our studies found that post-training infusions of M/B into the NAcc core and shell and post-training infusions of ANI into the NAcc core and shell had no effect on the mean difference score during PCA training sessions, suggesting that administration of M/B and ANI in the NAcc core and shell have no effect on the acquisition of PCA. However, there are several alternative interpretations of our data. It is possible that the doses of M/B and ANI used in these experiments were simply too low to have an effect on the acquisition of PCA behavior. This is unlikely, though, because the same dose of M/B had an effect on the expression of PCA, and the same doses of both drugs had an effect the acquisition of cued fear conditioning. Since all PCA training sessions were 90 min long, it is also possible that the cellular cascades underlying formation of the PCA memory had already begun before the end of the training session, and our post-training infusions might have occurred too late to have an effect on acquisition. However, this too is unlikely, at least in the case of ANI infusions. We have previously shown that post-training systemic administration of ANI impairs the consolidation of PCA {Blaiss, 2007 #313}, and these experiments show that it is clearly possible for post-training administration of ANI to affect learning in this paradigm.

Since systemic post-training infusions of ANI impair PCA behavior but intra-NAcc post-training infusions of ANI have no effect on PCA behavior, what is the

location of the new protein synthesis required for PCA acquisition? Surprisingly, there are few other brain regions that have been implicated in this behavior. However, the amygdala and the anterior cingulate cortex are two possible locations of plasticity underlying the acquisition of PCA. Post-training intra-amygdala infusions of *d*-amphetamine and a D_3 receptor agonist have been shown to enhance the acquisition of PCA, and lesions that functionally disconnect the basolateral amygdala and the NAcc result in impaired 2^{nd} order PCA behavior (although 1^{st} order PCA behavior is unaffected) (Hitchcott et al., 1997b; Hitchcott et al., 1997a). Lesions of the anterior cingulate cortex have been shown to impair the acquisition of an autoshaping task that is similar to the PCA task (Parkinson et al., 2000). The anatomy of these two brain regions also suggests that one of them could be the site of plasticity underlying the CS-US association. Both regions receive inputs from a variety of sensory areas that could convey information about the sensory properties of the CS (Cardinal et al., 2002a). In addition, multiple nuclei from the taste pathway could convey information about the US directly to the amygdala (Yamamoto, 2006).

Our experiments have also shown that pre-test infusions of M/B into the NAcc core and the NAcc shell impaired the difference scores exhibited during a PCA test session without impairing the rats' general activity level or motivation to consume the US. These data suggest that both the NAcc core and shell are necessary for the normal expression of the PCA behavior; however the core and shell appear to play slightly different roles in the expression of this behavior. After reversible inactivation of the NAcc core, rats exhibited a specific impairment in responding during the CS+, suggesting that they were impaired in their ability to express the CS-US association. After

reversible inactivation of the NAcc shell, however, rats exhibited a decrease in CS+ responding along with an increase in responding during the inter-trial interval. This suggests that the shell might be necessary to produce the behavioral efficiency seen after several training sessions in this task; without a functional NAcc shell, the rats appear unable to inhibit unnecessary behaviors.

The finding that neural activity in the NAcc core is necessary for the expression of PCA agrees with previous studies implicating the NAcc core in the performance of autoshaping behavior (Di Chiano et al., 2001; Cardinal et al., 2002b; Dalley et al., 2005) and PCA behavior (Parkinson et al., 1999). However, the finding the NAcc shell is involved in the expression of PCA contrasts with a previous study showing that excitotoxic lesions of the NAcc shell do not affect expression of conditioned approach behavior (Parkinson et al., 1999). Since muscimol and baclofen are known to diffuse across an area of brain tissue with a 1 mm radius (Martin and Ghez, 1999; Edeline et al., 2002), it is possible that infusions of M/B into the NAcc shell actually diffused into the core, causing the behavioral impairment seen in our study. This is unlikely, though, because our experiments have also shown that infusions of M/B into the NAcc shell do not affect the acquisition of cued fear conditioning, even though infusions of M/B into the NAcc core impair the acquisition of that task. Another explanation of the discrepancies between our results and the results of Parkinson, et al. (1999) is that, in the lesion study, the permanent loss of cells caused compensatory changes in other brain regions (including portions of the shell unaffected by the lesion), and this would be much less likely to be an issue with acute administration of M/B.

The role of the nucleus accumbens in cued fear conditioning

In contrast to the role of the NAcc in the acquisition of PCA, our results demonstrate that the NAcc core, but not the shell, is integrally involved in the acquisition of cued fear conditioning. Post-training infusions of M/B in the NAcc core, but not the shell, resulted in impaired freezing upon testing; rats that received infusions of M/B showed equivalent levels of freezing during the first two CS presentations, but they showed impaired levels of freezing compared to the vehicle group during later CS presentations. It could be possible to interpret this result as an enhancement of within-session extinction; however, this possibility is unlikely. M/B was administered on the training day but not the testing day, and therefore it is doubtful that M/B would be able to interfere with the short-term plasticity that would need to underlie within-session extinction. Because it has previously been shown that a weaker fear conditioning memory extinguishes at a faster rate than a stronger memory (Suzuki et al., 2004), it is more likely that post-training infusions of M/B impaired, but did not completely prevent, the learning of cued fear conditioning.

Although these results suggest that neural activity in the NAcc core is required for successful acquisition of cued fear conditioning, the neural activity could be required either to modulate plasticity occurring in other brain regions or to promote plasticity occurring within the core. However, the results from our experiment examining the effect of post-training inhibition of protein synthesis within the NAcc suggest that protein synthesis-dependent plasticity does indeed occur in the NAcc core. We found that post-training infusions of ANI in the NAcc core, but not the shell, resulted in impaired

freezing during a cued fear conditioning test session. These results suggest that protein synthesis is necessary within the NAcc core for the acquisition of cued fear conditioning, implying that protein synthesis-dependent plasticity occurring within the core is required for the acquisition of this task. Because the infusions of ANI (and the infusions of M/B, for that matter) were administered post-training, it is likely that these experimental manipulations impaired the consolidation of cued fear conditioning. However, in the absence of experiments investigating the effect of immediate post-training manipulations on short-term memory or the effect of delayed post-training manipulations on long-term memory, we are limited to concluding that the infusions of ANI and M/B affected the general acquisition of cued fear conditioning.

This finding that protein synthesis is required in the NAcc core for acquisition of this task does not easily fit into the most widely held model of the circuitry underlying cued fear conditioning. This model posits that the plasticity underlying the CS-US association occurs in the basolateral complex of the amygdala (BLA), and from there, information is thought to flow to the central nucleus of the amygdala (CeA) and finally out to the periacqueductal gray (PAG), a brainstem nucleus that is necessary for the freezing response (Fanselow and LeDoux, 1999; LeDoux, 2000, 2003; Kim and Jung, 2006). There is no evidence for a direct projection from the NAcc core to the amygdala, making it unlikely that the core acts as a modulatory input to this amygdala-based fear circuit (Zahm, 1999, 2000). However, both the NAcc core and shell receive projections from the BLA, and both subnuclei send efferent projections to the PAG (Usuda et al., 1998; Zahm, 1999, 2000). This anatomy suggests that there might be a parallel output circuit underlying cued fear conditioning, and this type of circuitry could explain our

results as well as the results of a recent study where lesions of the CeA only produced a mild impairment in fear conditioning (Koo et al., 2004).

Our results also showed that pre-test infusions of M/B into the NAcc core and shell had no effect on freezing behavior during a test session, suggesting that neural activity in the NAcc core and shell is not involved in the expression of cued fear conditioning. How can the NAcc core (and plasticity within the NAcc core) be required for the acquisition of cued fear conditioning but not its expression? It is possible to imagine a situation that would explain this situation. If the NAcc core is functioning in cued fear conditioning as part of a parallel output circuitry, perhaps the core must interact with a brain region in the other branch of the parallel circuit during acquisition in order to promote successful plasticity in both regions. In this case, acquisition would be impaired if either branch of the circuit was impaired. However, if the parallel branches of the circuit were able to function independently after the acquisition and consolidation processes were completed, then impairment of one branch of the circuit would still leave the behavior intact.

Conclusions

Our results suggest that, unlike autoshaping and some forms of instrumental conditioning, the NAcc is minimally involved in the acquisition of the PCA association and not the site of any protein synthesis-dependent plasticity underlying the association. We have also shown that neural activity in the NAcc core and shell necessary for the normal expression of PCA behavior, but the core and shell appear to play different roles in PCA expression. In addition, our results suggest that although the NAcc does not

appear to be involved in the normal expression of cued fear conditioning, the NAcc core (but not the shell) is integrally involved in the acquisition of this aversive Pavlovian association. This suggests that the circuitry underlying the acquisition of cued fear conditioning is broader than generally thought. Taken as a whole (see Figure 7 for a summary), our results suggest that the NAcc plays different roles in the acquisition and expression of appetitive and aversive Pavlovian conditioning.

References

Balleine BW (2005) Neural bases of food-seeking: affect, arousal and reward in corticostiatolimbic circuits. Physiology and Behavior 86:717-730.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002a) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neuroscience and Biobehavioral Reviews 26:321-352.

Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ (2001) Impulsive choice induced in rats by lesions of the nucleus accumbens core. Science 2929:2499-2501.

Cardinal RN, Parkinson JA, Lachenal G, Halkerston KM, Rudarakanchana N, Hall J, Morrison CH, Howes SR, Robbins TW, Everitt BJ (2002b) Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. Behavioral Neuroscience 116:553-567.

Cassaday HJ, Horsley RR, Norman C (2005) Electrolytic lesions to nucleus accumbens core and shell have dissociable effects on conditioning to discrete and contextual cues in aversive and appetitive procedures respectively. Behavioural Brain Research 160:222-235.

Corbit LH, Muir JL, Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: evidence of a functional dissociation between accumbens core and shell. Journal of Neuroscience 21:3251-3260.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and

NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci USA 102:6189-6194.

Di Chiano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/Kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of Pavlovian approach behavior. J Neurosci 21:9471-9477.

Edeline J-M, Hars B, Hennevin E, Cotillon N (2002) Muscimol diffusion after intracerebral microinjections: a reevaluation based on electrophysiological and autoradiographic quantifications. Neurobiology of learning and memory 78:100-124.

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward: the role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 877:412-438.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Ferretti V, Florian C, Costantini VJA, Roullet P, Rinaldi A, De Leonibus E, Oliverio A, Mele A (2005) Co-activation of glutamate and dopamine receptors within the nucleus accumbens is required for spatial memory consolidation in mice. Psychopharmacology 179:108-116.

Hall J, Parkinson JA, Connor TM, Dickinson A, Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. European Journal of Neuroscience 13:1984-1992.

Haralambous T, Westbrook RF (1999) An infusion of bupivacaine into the nucleus accumbens disrupts the acquisition but not the expression of contextual fear conditioning. Behavioral Neuroscience 113:925-940.

Hernandez PJ, Sadeghian K, Kelley AE (2002) Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. Nature Neuroscience 5:1327-1331.

Hitchcott PK, Harmer CJ, Phillips GD (1997a) Enhanced acquisition of discriminative approach following intra-amygdala d-amphetamine. Psychopharmacology 132:237-246.

Hitchcott PK, Bonardi CMT, Phillips GD (1997b) Enhanced stimulus-reward learning by intra-amygdala administration of a D_3 dopamine receptor agonist. Pyschopharmacology 133:240-248.

Jackson DM, Anden N-E, Dahlström A (1975) A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. Psychopharmacologia 45:139-149.

Kelley AE (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neuroscience and Biobehavioral Reviews 27:765-776.

Kelly PH, Iversen SD (1976) Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. European Journal of Pharmacology 40:45-56.

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research 94:507-522.

Kim JJ, Jung MW (2006) Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neuroscience and Biobehavioral Reviews 30:188-202.

Koo JW, Han JS, Kim JJ (2004) Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. J Neurosci 24:7654-7662.

LeDoux JE (2000) Emotion circuits in the brain. Annual Reviews in Neuroscience 23:155-184.

LeDoux JE (2003) The emotional brain, fear, and the amygdala. Cellular and Molecular Neurobiology 23:727-738.

Levita L, Dalley JW, Robbins TW (2002) Disruption of Pavlovian contextual conditioning by excitotoxic lesions of the nucleus accumbens core. Behavioral Neuroscience 116:539-552.

Martin JH, Ghez C (1999) Pharmacological inactivation in the analysis of the central control of movement. Journal of Neuroscience Methods 86:145-159.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. Journal of Neuroscience 21:8655-8663.

Meiri N, Rosenblum K (1998) Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. Brain Research 789:48-55.

Mogenson GJ (1987) Limbic-motor integration. In: Progress in Psychobiology and Physiological Psychology (Epstein AN, ed), pp 117-170. New York: Academic Press.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Progress in Neurobiology 14:69-97.

Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. Behavioral Neuroscience 114:42-63.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by d-amphetamine. J Neurosci 19:2401-2411.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, fourth edition. New York: Academic Press.

Phillips GD, Setzu E, Hitchcott PK (2003) Facilitation of Appetitive Pavlovian Conditioning by *d*-Amphetamine in the Shell, but Not the Core, of the Nucleus Accumbens. Behavioral Neuroscience 117:675-684.

Pijnenburg AJJ, Woodruff GN, van Rossum JM (1973) Ergometrine induced locomotor activity following intra-cerebral injection into the nucleus accumbens. Brain Research 59:289-302.

Rosenblum K, Meiri N, Dudai Y (1993) Taste memory: the role of protein synthesis in gustatory cortex. Behavioral and Neural Biology 59:49-56.

Schoenbaum G, Setlow B (2003) Lesions of nucleus accumbens disrupt learning about aversive outcomes. Journal of Neuroscience 23:9833-9841.

Setlow B (1997) The nucleus accumbens and learning and memory. Journal of Neuroscience Research 49:515-521.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787-4795.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Research 797:73-93.

Yamamoto T (2006) Neural substrates for the processing of cognitive and affective aspects of taste in the brain. Arch Histol Cytol 69:243-255.

Zahm DS (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Annals of the New York Academy of Sciences 877:113-128.

Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neuroscience and Biobehavioral Reviews 24:85-105.

Figure Legends

Figure 1. Effect of protein synthesis inhibition within the NAcc on the acquisition of PCA behavior

a) General experimental design

b) Mean difference score ± SEM during PCA training sessions for rats that received infusions into the NAcc core (Vehicle: n=12; Ani: n=11)

c) Mean difference score ± SEM during PCA training sessions for rats that received infusions into the NAcc shell (Vehicle: n=6; Ani: n=6)

Figure 2. Effect of reversible inactivation of the NAcc on the acquisition of PCA behavior

a) General experimental design

b) Mean difference score ± SEM during PCA training sessions for rats that received infusions into the NAcc core (Vehicle: n=6; M/B: n=7)

c) Mean difference score ± SEM during PCA training sessions for rats that received infusions into the NAcc shell (Vehicle: n=7; M/B: n=8)

Figure 3. Effect of reversible inactivation of the NAcc on the expression of PCA behavior

a) General experimental design

b) Mean difference score (± SEM) during PCA training and test sessions for rats that received infusions into the NAcc core (n=15) *P<.002

c) Mean difference score (± SEM) during PCA training and test sessions for rats that received infusions into the NAcc shell (n=8) *P<.001

d) and e) Port entries exhibited by representative rats. The top half of each graph is a perievent raster where each line represents a separate trial during the training/test session, each tick mark represents a single port entry, black circles represent the onset of the CS+, and black triangles represent the onset of the pump that delivered the sucrose reward. The bottom half of each graph is a perievent histogram showing the number of port entries (during 2 sec bins) made during all trials of the training/test session. The zero time point represents the onset of the CS+. The graph on the left represents the port entries made during the first training session; the graph in the middle represents the port entries made during the test session where rats had been infused with vehicle; and the graph on the right represents the port entries made during the test session where rats had been infused with M/B. **d)** Port entries made by a representative rat that received infusions in the NAcc core. **e)** Port entries made by a representative rat that received infusions in the NAcc shell.

f) Mean number of port entries (± SEM) during the PCA test sessions *P<.009

g) Mean number of trials (± SEM) in the PCA test sessions during which there was a response to the US delivery

Figure 4. Effect of protein synthesis inhibition within the NAcc on the acquisition of cued fear conditioning

a) General experimental design

b) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc core (Vehicle: n=8; Ani: n=7) *P<.05

c) Mean number of line crossings (± SEM) during the habituation period of the test session for the rats that received infusions into the NAcc core

d) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc shell (Vehicle: n=11; Ani: n=7)

Figure 5. Effect of reversible inactivation of the NAcc on the acquisition of cued fear conditioning

a) General experimental design

b) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc core (Vehicle: n=12; M/B: n=9) *P<.05

c) Mean number of line crossings (± SEM) during the habituation period of the test session for the rats that received infusions into the NAcc core

d) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc shell (Vehicle: n=8; M/B: n=7)

Figure 6. Effect of reversible inactivation of the NAcc on the expression of cued fear conditioning

a) General experimental design

b) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc core (Vehicle: n=8; M/B: n=7)

c) Mean percent of time spent freezing (± SEM) during the test session for the rats that received infusions into the NAcc shell (Vehicle: n=7; M/B: n=6)

Figure 7. Summary of the results of experiments investigating the role of the NAcc core and shell in appetitive and aversive Pavlovian conditioning.

Figure 1

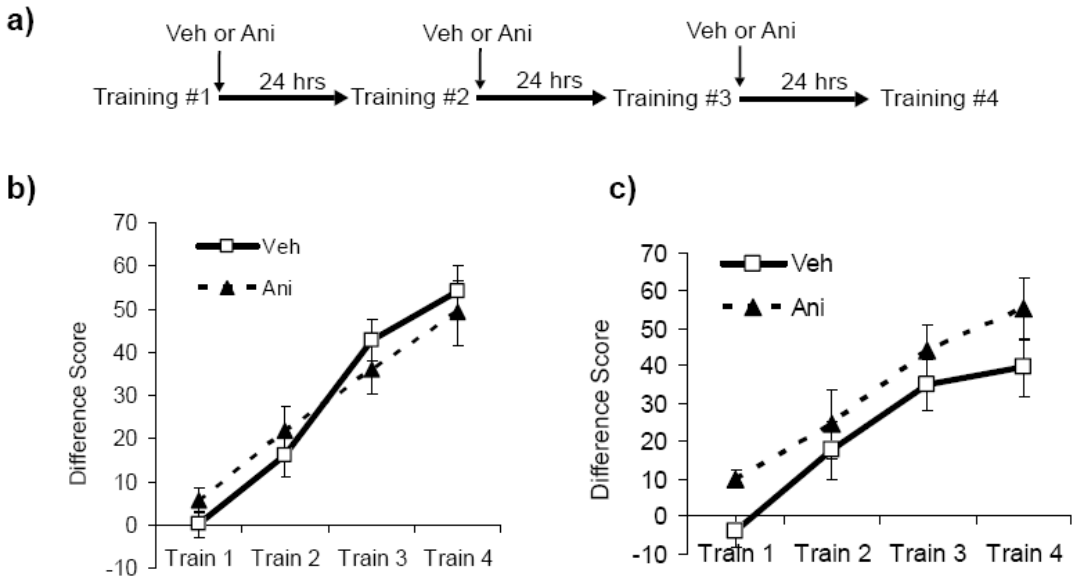

a)
Veh or Ani
Veh or Ani
Veh or Ani
Training #1
24 hrs
Training #2
24 hrs
Training #3
24 hrs
Training #4
b)
Difference Score
Veh
Ani
70
60
50
40
30
20
10
0
-10
Train 1
Train 2
Train 3
Train 4
c)
Difference Score
Veh
Ani
70
60
50
40
30
20
10
0
-10
Train 1
Train 2
Train 3
Train 4

Figure 2

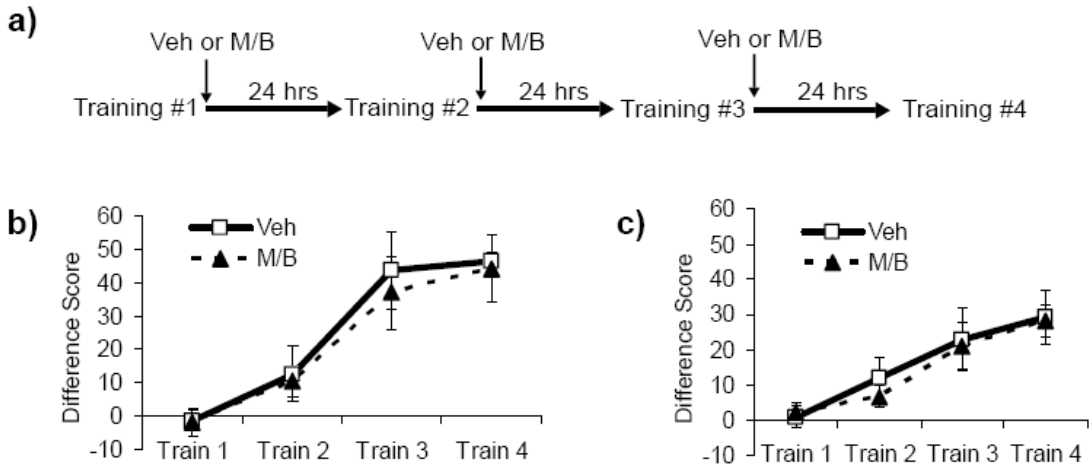

a)
Veh or M/B
Veh or M/B
Veh or M/B
Training #1
24 hrs
Training #2
24 hrs
Training #3
24 hrs
Training #4
b)
Veh
M/B
Difference Score
60
50
40
30
20
10
0
-10
Train 1
Train 2
Train 3
Train 4
c)
Veh
M/B
Difference Score
60
50
40
30
20
10
0
-10
Train 1
Train 2
Train 3
Train 4

Figure 3

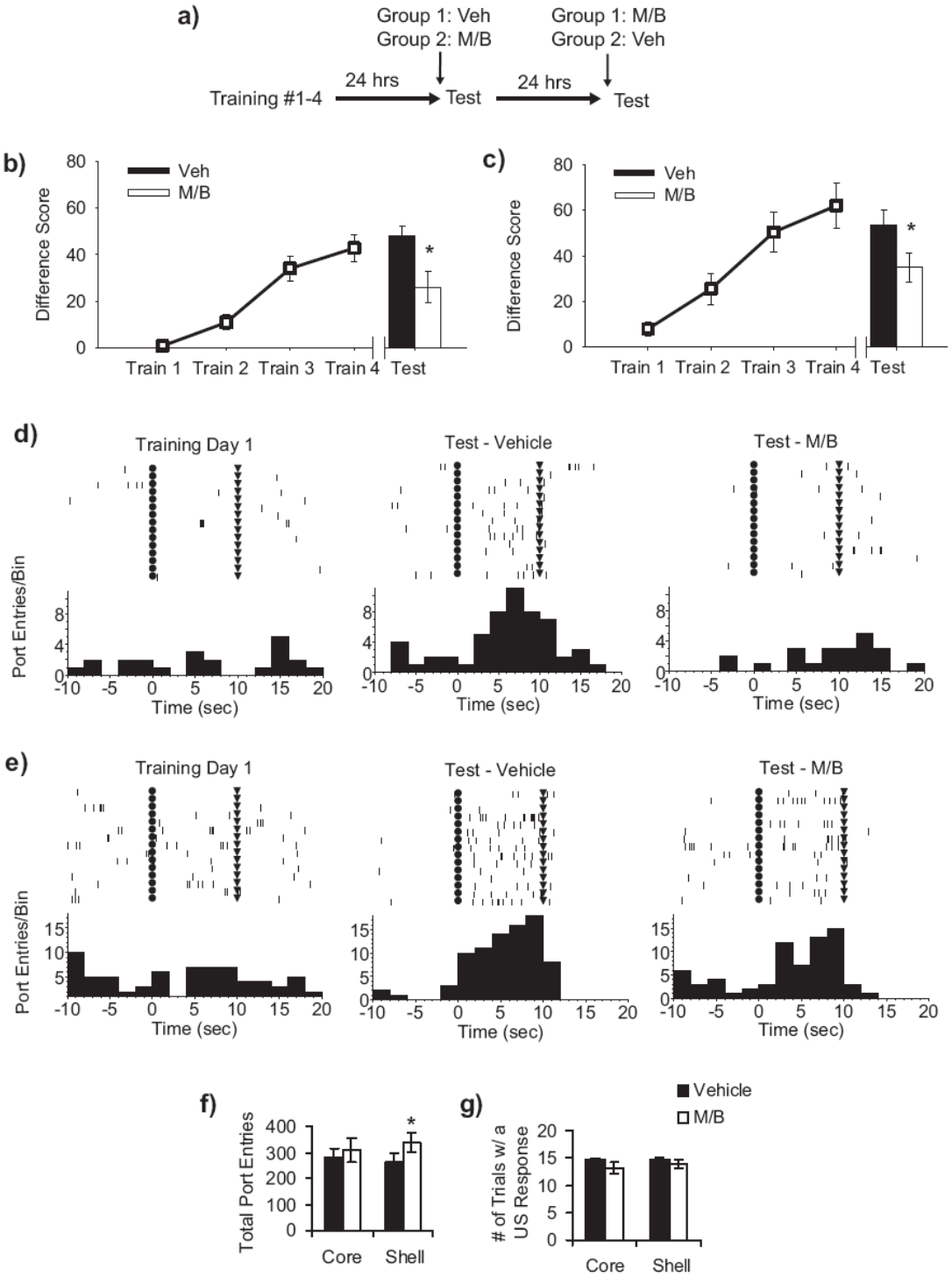

a)
Group 1: Veh
Group 2: M/B
Group 1: M/B
Group 2: Veh
24 hrs
24 hrs
Training #1-4
Test
Test
b)
Veh
M/B
Difference Score
Train 1 Train 2 Train 3 Train 4 Test
c)
Veh
M/B
Difference Score
Train 1 Train 2 Train 3 Train 4 Test
d)
Training Day 1
Test - Vehicle
Test - M/B
Port Entries/Bin
Time (sec)
e)
Training Day 1
Test - Vehicle
Test - M/B
Port Entries/Bin
Time (sec)
f)
Total Port Entries
Core
Shell
g)
Vehicle
M/B
# of Trials w/ a US Response
Core
Shell

Figure 4

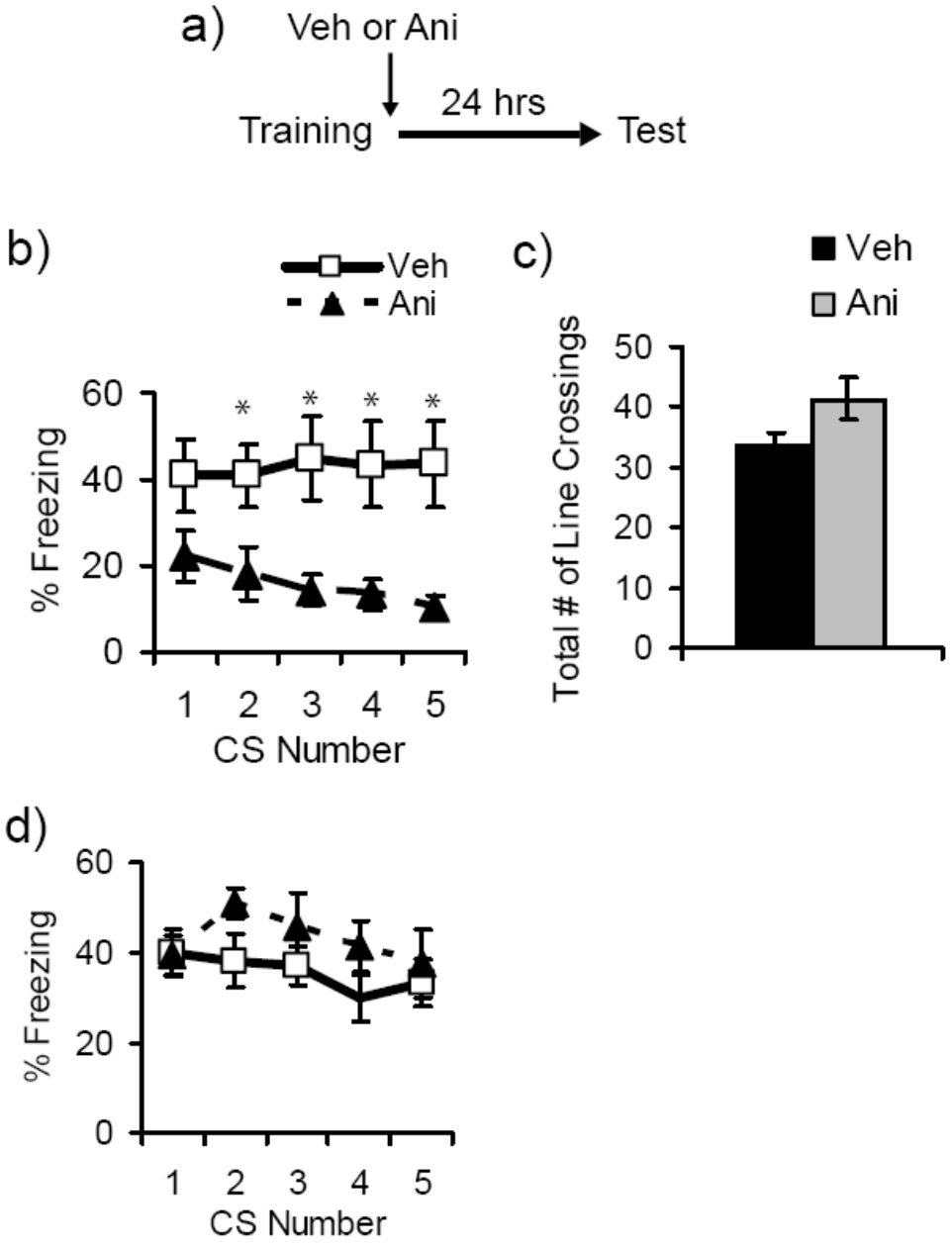

a)
Veh or Ani
Training
24 hrs
Test
b)
Veh
Ani
% Freezing
60
40
20
0
*
*
*
*
1
2
3
4
5
CS Number
c)
Veh
Ani
Total # of Line Crossings
50
40
30
20
10
0
d)
% Freezing
60
40
20
0
1
2
3
4
5
CS Number

Figure 5

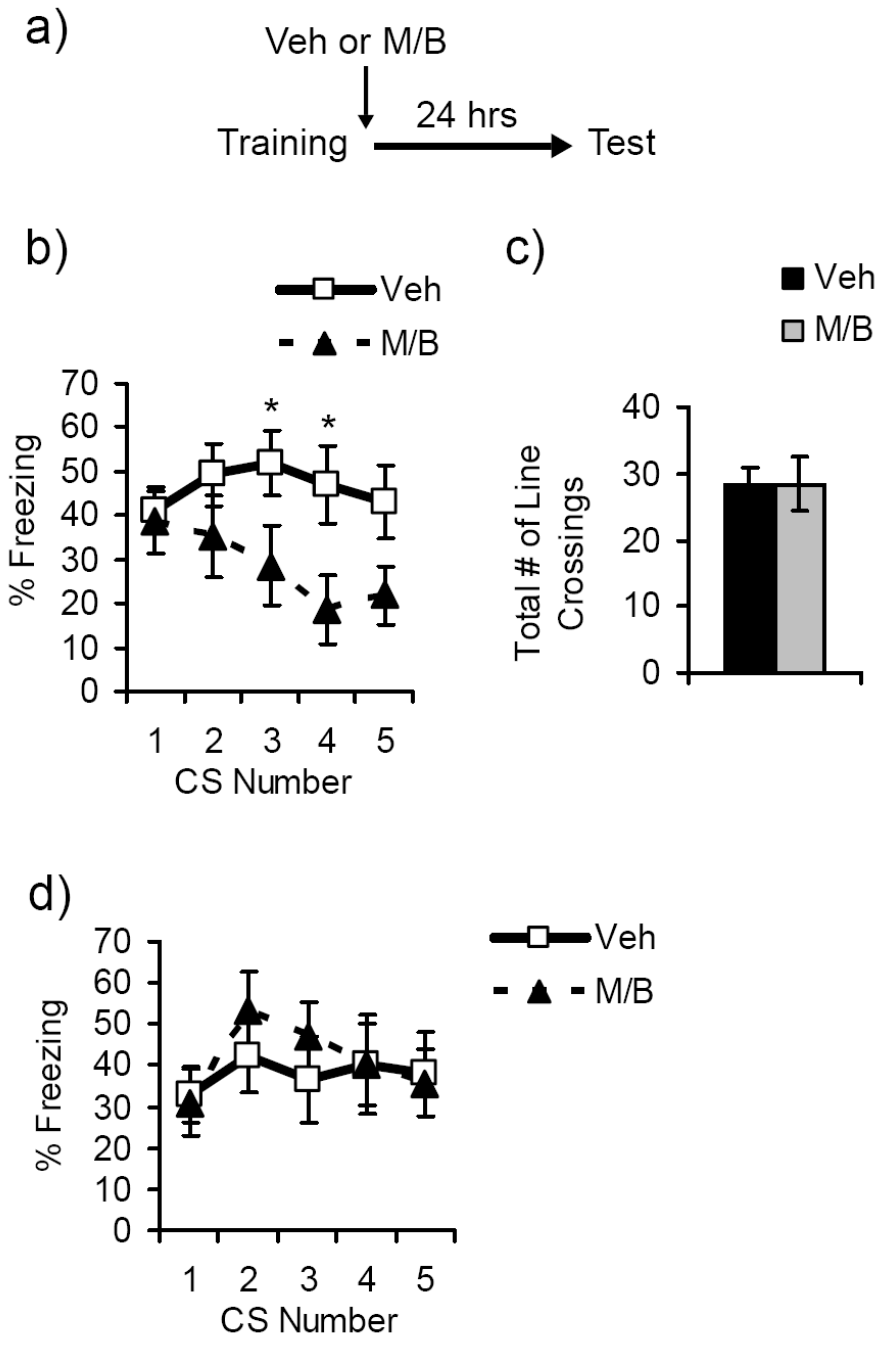

a)
Veh or M/B
Training
24 hrs
Test
b)
Veh
M/B
% Freezing
70
60
50
40
30
20
10
0
*
*
1 2 3 4 5
CS Number
c)
Veh
M/B
Total # of Line Crossings
40
30
20
10
0
d)
Veh
M/B
% Freezing
70
60
50
40
30
20
10
0
1 2 3 4 5
CS Number

Figure 6

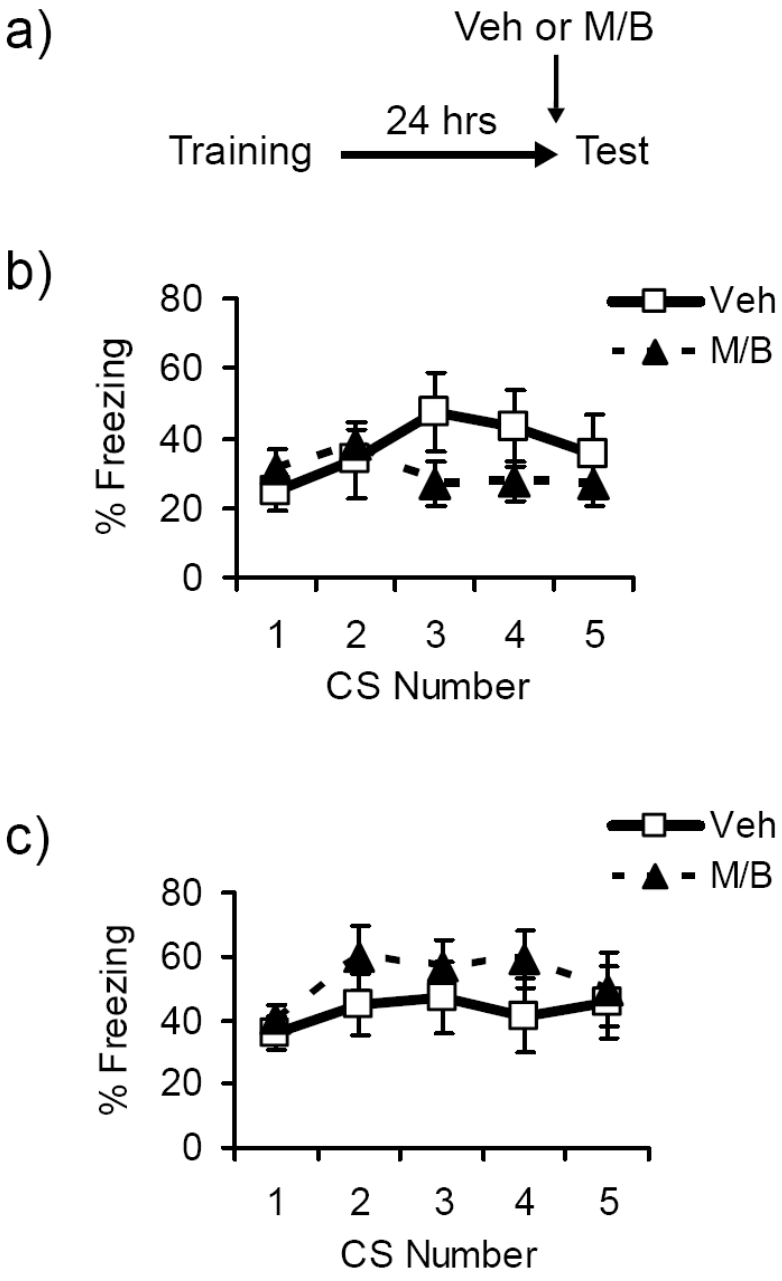
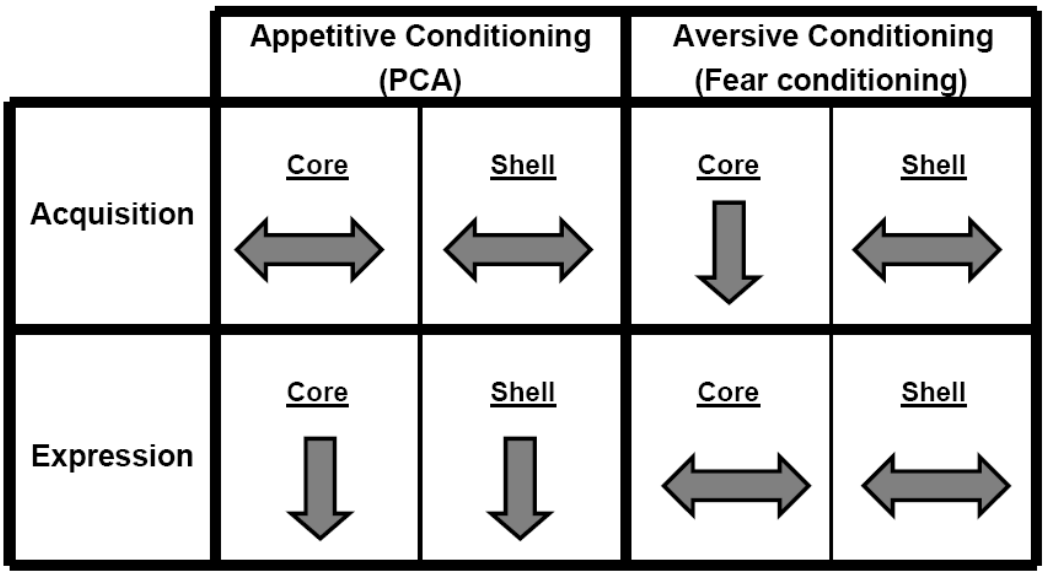

a)
Veh or M/B
24 hrs
Training
Test
b)
Veh
M/B
% Freezing
80
60
40
20
0
1
2
3
4
5
CS Number
c)
Veh
M/B
% Freezing
80
60
40
20
0
1
2
3
4
5
CS Number

Figure 7


Appetitive Conditioning (PCA)
Aversive Conditioning (Fear conditioning)
Acquisition
Expression
Core
Shell
Core
Shell
Core
Shell
Core
Shell

Supplementary Figure Legends

Supplementary Figure 1. Schematic representation of cannula placements for the experiment examining the effect of protein synthesis inhibition in the NAcc on the acquisition of PCA.

a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

Supplementary Figure 2. Schematic representation of cannula placements for the experiment examining the effect of reversible inactivation of the NAcc on the acquisition of PCA.

a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

Supplementary Figure 3. Schematic representation of cannula placements for the experiment examining the effect of reversible inactivation of the NAcc on the expression of PCA.

a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

Supplementary Figure 4. Schematic representation of cannula placements for the experiment examining the effect of protein synthesis inhibition in the NAcc on the acquisition of cued fear conditioning.

a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

Supplementary Figure 5. Schematic representation of cannula placements for the experiment examining the effect of reversible inactivation of the NAcc on the acquisition of cued fear conditioning.

a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

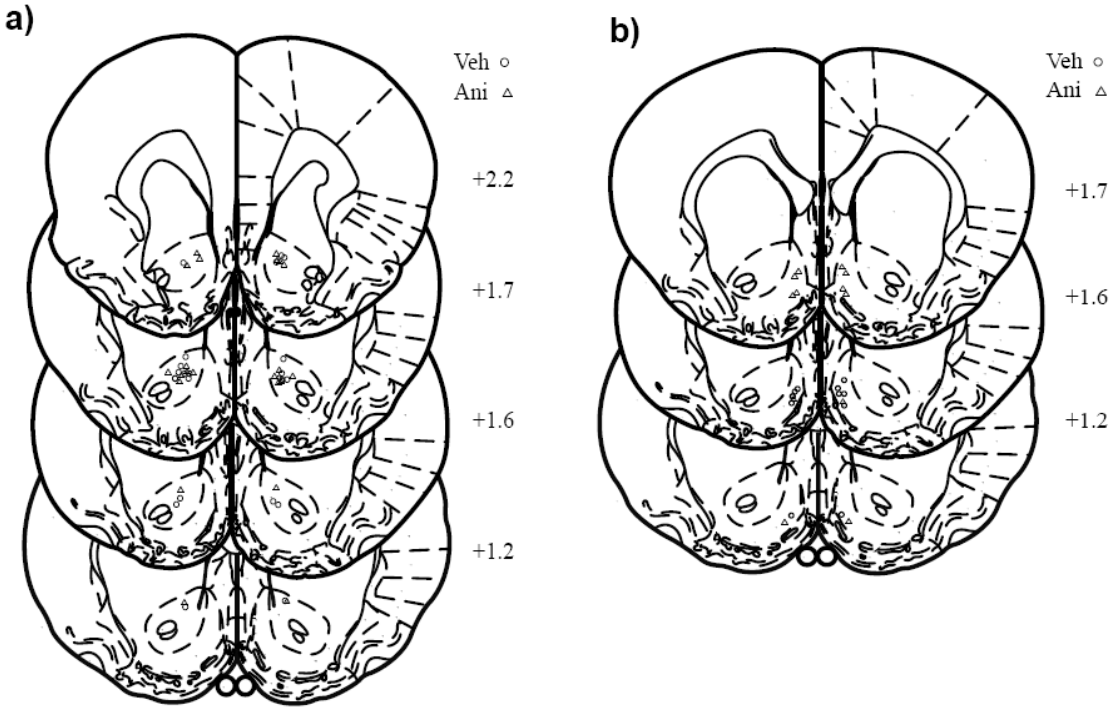
b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

Supplementary Figure 6. Schematic representation of cannula placements for the experiment examining the effect of reversible inactivation of the NAcc on the expression of cued fear conditioning.

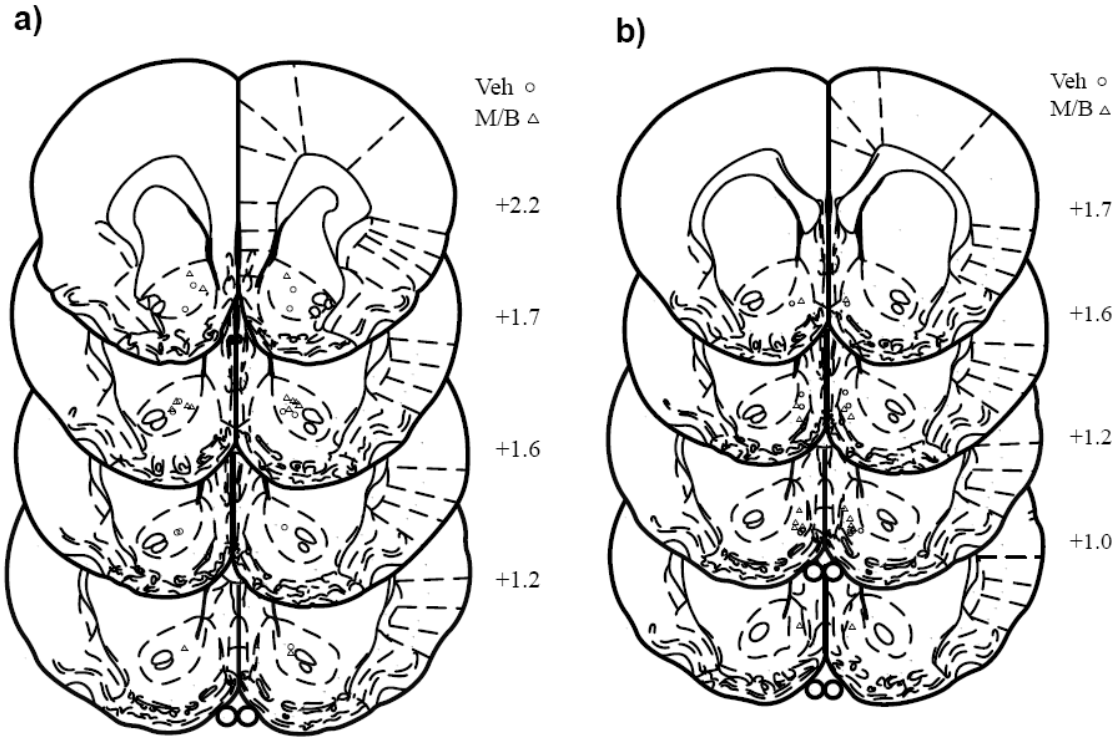
a) Cannula placements for rats that received infusions into the NAcc core. Coordinates are A/P from Bregma.

b) Cannula placements for rats that received infusions into the NAcc shell. Coordinates are A/P from Bregma.

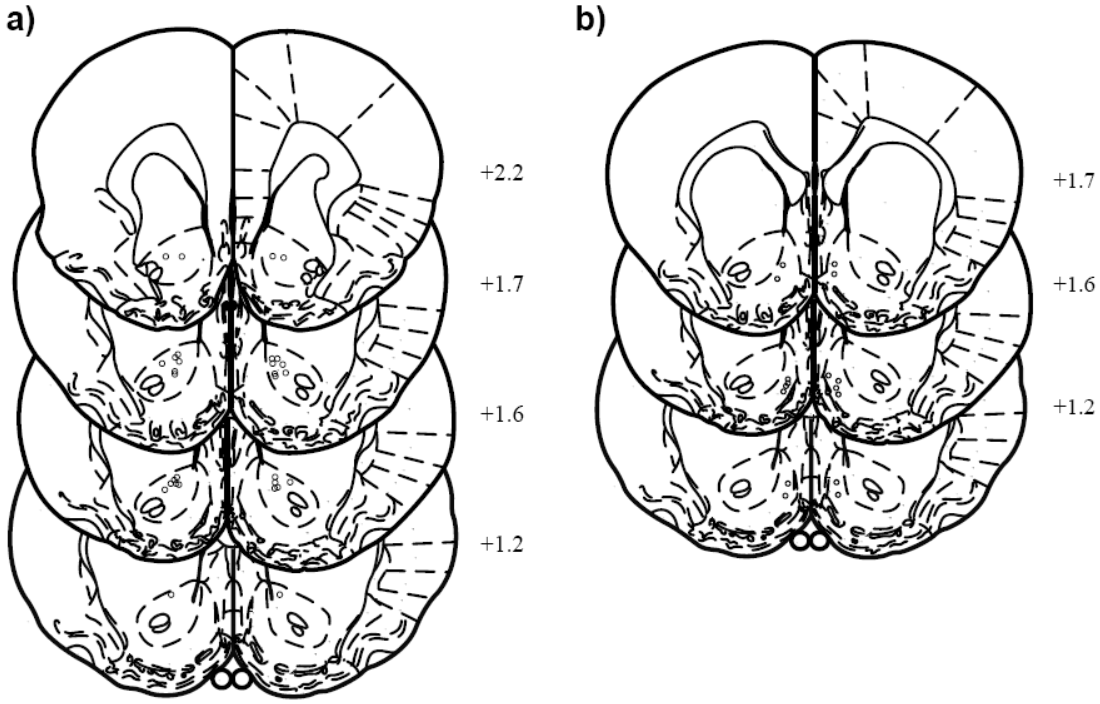
Supplementary Figure 1



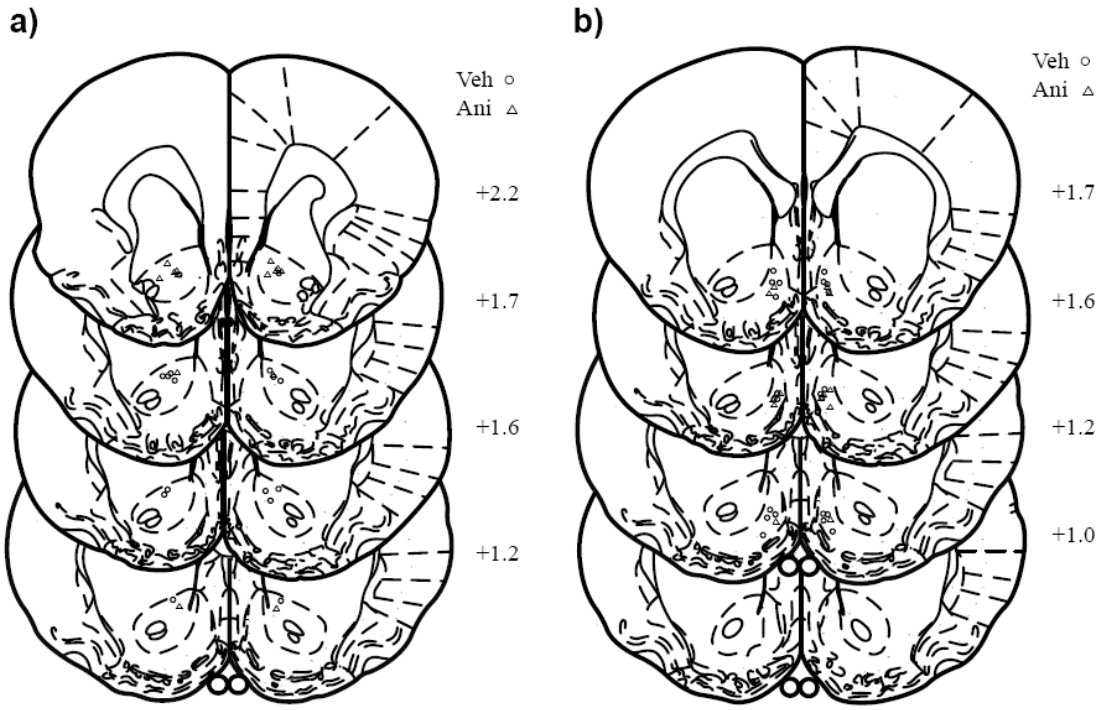
Supplementary Figure 2



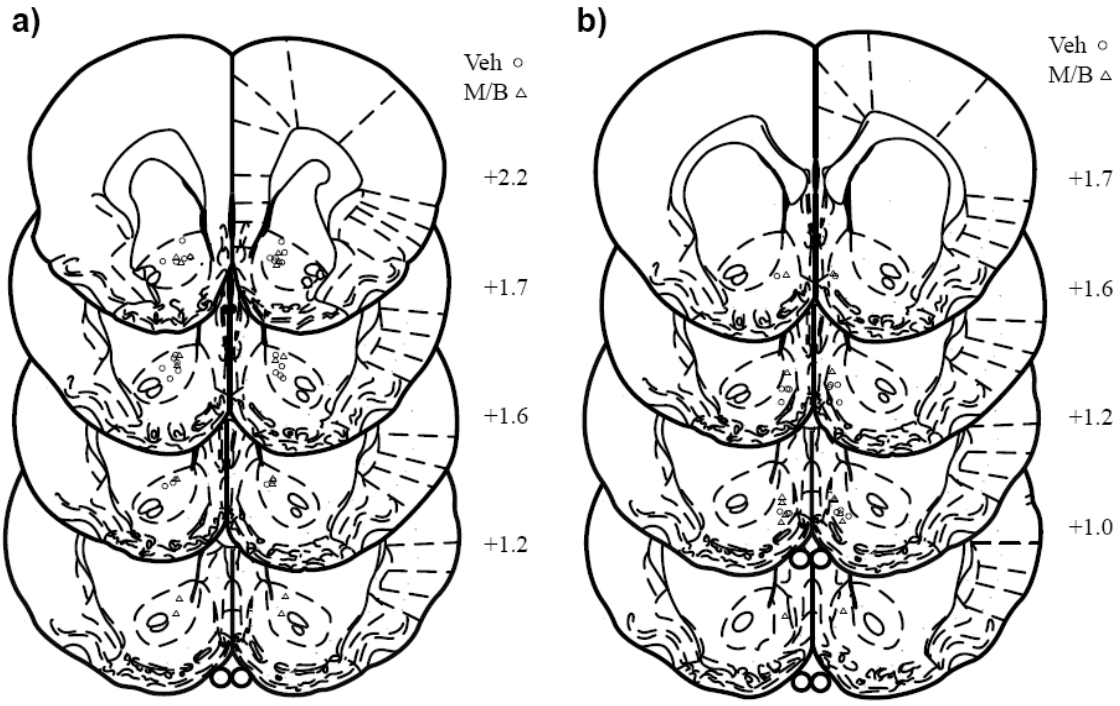
Supplementary Figure 3



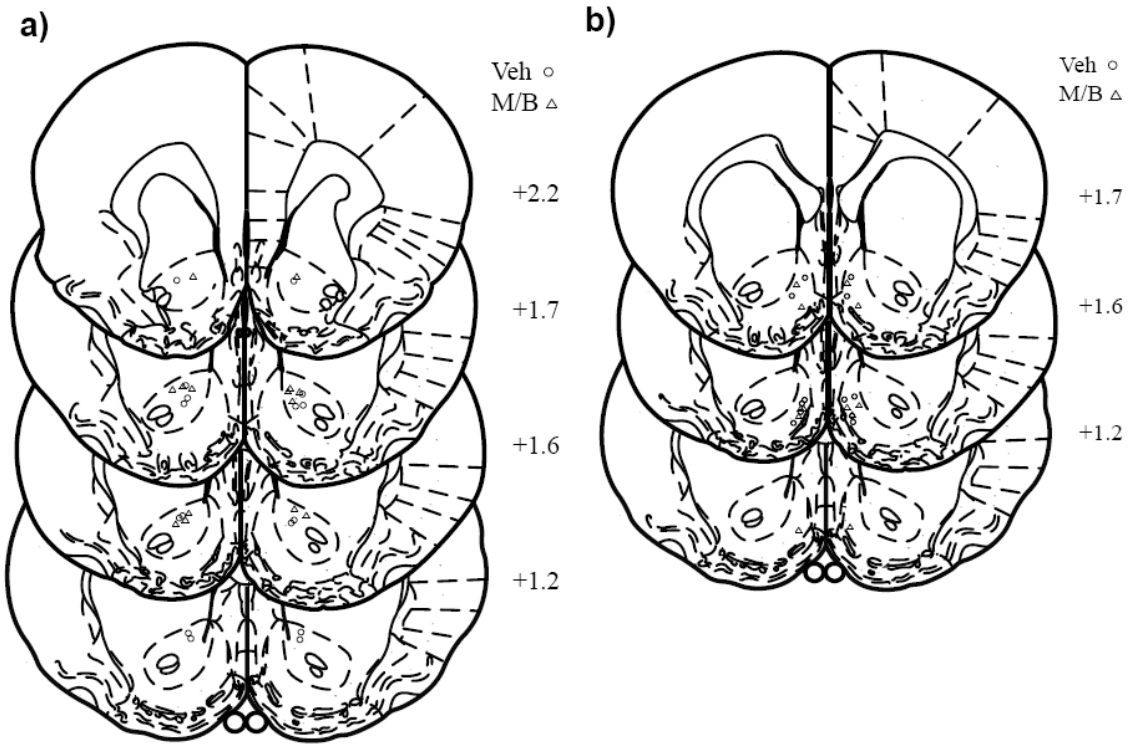
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Chapter Five:
General Discussion

Summary

There are several main findings from the experiments in this dissertation. In experiments discussed in Chapters Two and Three, systemic injections of amphetamine (AMPH) immediately, but not 6 hrs, after training sessions resulted in an enhanced preference in the morphine conditioned place preference (mCPP) task and an enhanced ability to discriminate between the conditioned stimulus (CS+) and the neutral stimulus (CS-) in the Pavlovian conditioned approach (PCA) task. This suggests that AMPH enhances the consolidation of both mCPP and PCA, and these results demonstrate that the consolidation of associations formed in appetitive Pavlovian conditioning tasks can be enhanced in a manner similar to other types of learning. These results also demonstrate that a drug of abuse can enhance multiple forms of addiction-related learning.

The results from Chapter Three also show that systemic injections of the protein synthesis inhibitor anisomycin (ANI) administered immediately, but not 3 hrs, after training impairs the ability of rats to discriminate between the CS+ and CS- in the PCA task, and this impairment is not due to a general effect of the drug on motivation. These data imply that, like multiple other forms of learning and memory, the memory for the PCA association requires the synthesis of new proteins. However, the experiments in Chapter Four suggest that the new protein synthesis required for the consolidation of this task does not occur in either the nucleus accumbens (NAcc) core or shell. Post-training infusions of ANI into the NAcc core and shell had no effect on PCA behavior. In

addition, post-training reversible inactivation of the NAcc core and shell also had no effect on PCA behavior, suggesting that the NAcc as a whole is not involved in the acquisition of the Pavlovian associations underlying the PCA task. Therefore, the location of the new protein synthesis required for the consolidation of PCA remains unknown.

In addition to the investigation of consolidation, the experiments in Chapters Two and Three also investigated the ability of AMPH and ANI to modulate the reconsolidation of appetitive Pavlovian conditioning. Systemic injections of AMPH immediately, but not 6 hrs, after memory reactivation resulted in an enhanced preference in the mCPP task, but injections of AMPH in the absence of the behavioral memory reactivation had no effect on mCPP behavior. These data suggest that AMPH enhances the reconsolidation of mCPP. In contrast, systemic injections of AMPH after memory reactivation had no effect on PCA behavior, and post-reactivation systemic injections of ANI also had no effect on PCA behavior. Post-reactivation injections of AMPH and ANI still had no effect on PCA behavior even when multiple variations in the behavioral protocol were used in the experiment, suggesting that the PCA association exhibits limited lability after memory reactivation.

The experiments in Chapter Four investigated the role of the NAcc core and shell in the acquisition and expression of PCA and compared it to the role of the NAcc core and shell in the acquisition and expression in cued fear conditioning, a form of aversive Pavlovian conditioning. As described above, the results from these experiments suggest that NAcc as a whole is not involved in the acquisition of PCA. In contrast, both post-training reversible inactivation and post-training inhibition of protein synthesis in the

NAcc core (but not the shell) impaired the acquisition of cued fear conditioning, suggesting that unlike the acquisition of PCA, protein synthesis-dependent plasticity is required in the NAcc core for the acquisition of this type of aversive Pavlovian conditioning. In addition, reversible inactivation of both the NAcc core and shell impaired the expression of PCA, but reversible inactivation of the NAcc core and shell had no effect on the expression of cued fear conditioning. These results suggest that the NAcc is differentially involved in the acquisition and expression of appetitive and aversive Pavlovian conditioning.

The effect of amphetamine on the consolidation of appetitive Pavlovian conditioning

The results of experiments in Chapters Two and Three demonstrate that AMPH enhances the consolidation of two forms of appetitive Pavlovian conditioning: mCPP and PCA. This agrees with a large literature showing that AMPH enhances a wide variety of different learning and memory tasks (Doty and Doty, 1966; McGaugh, 1966; Kulkarni, 1968; Krivanek and McGaugh, 1969; Strupp et al., 1991; Janak and Martinez, 1992; Soetens et al., 1993; Soetens et al., 1995; Brown et al., 2000).

However, these results do not provide information about the location in the brain where is AMPH acting to enhance the consolidation of mCPP and PCA. Because the basolateral complex of the amygdala (BLA) has been shown to be generally involved in the consolidation of CPP (Hsu et al., 2002; Schroeder and Packard, 2002), it is possible that AMPH might be acting in this nucleus. In addition, studies have suggested that AMPH can act in multiple brain regions to modulate the acquisition of PCA; multiple

infusions of AMPH into the NAcc shell and into the amygdala enhance the acquisition of PCA (Hitchcott et al., 1997b; Phillips et al., 2003)

In the case of both mCPP and PCA, however, the mechanism through which AMPH acts to enhance consolidation remains unclear. As discussed in previous chapters, studies of other types of learning and memory have suggested that both the dopaminergic and noradrenergic systems are involved in the enhancing effect of AMPH on consolidation (Lee and Ma, 1995; Brown et al., 2000; Fenu and Di Chiara, 2003), and it is possible that these neurotransmitter systems are responsible for the enhancing effect of AMPH in the mCPP and PCA tasks.

Further study of the effect of AMPH on the consolidation of these types of appetitive Pavlovian conditioning tasks would help us to better understand the manner in which drugs of abuse modulate types of learning that contribute to the pathological development of drug-seeking behavior. It has been hypothesized that the transition from casual drug use to addiction occurs in part because drugs of abuse have an ability to distort the functioning of normal learning and memory pathways in a way that enhances addiction-related learning and ultimately contributes to pathological drug-seeking behavior (Berke and Hyman, 2000; Ammassari-Teule, 2001). The experiments investigating the effect of AMPH on consolidation show an example of a similar situation; the results of these experiments demonstrate that a drug of abuse has the ability to enhance the association between a cue or context (conditioned stimulus, CS) and a reward (unconditioned stimulus, US), thereby enhancing the ability of the CS to elicit reward-seeking behavior. Future studies of the mechanism underlying this memory

enhancing effect of AMPH could contribute to a better understanding of the ways in which drugs of abuse modulate normal pathways involved in learning and memory.

The effect of protein synthesis inhibition on the consolidation of appetitive Pavlovian conditioning

In Chapter Three, post-training systemic infusions of ANI were shown to impair the consolidation of PCA, suggesting that protein synthesis is required for the consolidation of this task. Systemic injections of a protein synthesis inhibitor have also been shown to impair the consolidation of mCPP (Milekic et al., 2006), suggesting that protein synthesis is generally required for the consolidation of multiple types of appetitive Pavlovian conditioning.

Which brain regions require protein synthesis for the consolidation of PCA? From the results of the experiments detailed in Chapter Four, it appears that even though the NAcc shell and core are required for normal expression of PCA, new protein synthesis is not required in either of these subnuclei for PCA consolidation. There is a dearth of evidence in the literature that would point to other candidate brain regions, but two possible locations of plasticity underlying the CS-US association could be the BLA or the anterior cingulate cortex (AntCing). Both the BLA and AntCing receive afferent projections from brain regions (such as various sensory cortices and the ventral tegmental area) that could convey information about both the CS and the sucrose US (Zahm, 2000; Cardinal et al., 2002a), and therefore these regions are possible locations of convergence of information about the CS and US (see Figure 1). In addition, both of these regions project directly to the NAcc, which the experiments in Chapter Four implicated in the

expression of PCA (Zahm, 2000; Cardinal et al., 2002a; Voorn et al., 2004). Lesions disconnecting the BLA and the NAcc impair second order PCA (Setlow et al., 2002), but while there is evidence suggesting that the BLA plays a modulatory role in the acquisition of first order PCA, there is also evidence that this nucleus is not involved in the acquisition of this task (Hitchcott et al., 1997a; Setlow et al., 2002). There is also evidence that the AntCing is necessary for autoshaping, another form of appetitive Pavlovian conditioning (Parkinson et al., 2000).

However, it should be noted that there are important distinctions between the autoshaping and PCA tasks that could affect the neural circuitry involved in these behaviors. In the autoshaping paradigm, rats are trained to associate the presentation of a CS with the delivery of a food reward, but the autoshaping task examines CS-induced approach to the source of the CS (i.e. approach to an LED display that presents a visual CS) as a behavioral measure of conditioning (Parkinson et al., 2000; Di Chiano et al., 2001; Cardinal et al., 2002b; Dalley et al., 2005). In contrast, the PCA task examines CS-induced approach to the US port as a behavioral measure of conditioning. Differences in the neural circuitry underlying autoshaping and conditioned orienting, another form of appetitive Pavlovian conditioning that uses a third distinct behavioral measure as an outcome of conditioning, have already been shown, and it has been suggested that these tasks have different circuitry to mediate the different motor outputs (Everitt et al., 1999; Setlow et al., 2003). Additional studies have shown differences in role of the amygdala in the conditioned orienting and PCA tasks (Setlow et al., 2002; Setlow et al., 2003). Therefore, the literature investigating the neural circuitry of other appetitive Pavlovian

conditioning tasks such as autoshaping and conditioned orienting should be considered with caution when trying to identify candidate brain regions for the study of PCA.

Why might different (although overlapping) neural circuits be necessary for these different appetitive Pavlovian conditioning tasks? PCA, autoshaping, and conditioned orienting all represent different conditioned responses to the same environmental stimuli. In the rat's natural environment, however, each of these separate conditioned responses might serve a distinct purpose. For instance, conditioned approach behavior could help animals develop a competitive advantage in acquiring food and other desirable rewards, and conditioned orienting could ensure that animals are paying attention to the CS in case there is any new or altered information that needs to be incorporated into the previously learned association. Since these behavioral responses serve different purposes in the natural environment, it is possible that they developed at different times in evolutionary history and therefore utilized different neural circuits.

The reconsolidation of appetitive Pavlovian conditioning

Experiments in Chapters Two and Three investigated the effect of AMPH and ANI on the reconsolidation of appetitive Pavlovian conditioning. The results suggest that AMPH enhances the reconsolidation of mCPP but does not seem to affect the reconsolidation of PCA. Systemic injections of ANI also had no effect on the reconsolidation of PCA, suggesting that protein synthesis is not required for the reconsolidation of PCA. Other groups have examined the effect of protein synthesis inhibition on the reconsolidation of mCPP, and they have found different results. Two studies found that systemic inhibition of protein synthesis impaired the reconsolidation of

mCPP if the memory reactivation session included both morphine administration and exposure to the context (Milekic et al., 2006; Valjent et al., 2006), but two studies found that systemic inhibition of protein synthesis had no effect on the reconsolidation of mCPP (Yim et al., 2006; Robinson and Franklin, 2007). These data suggest that although mCPP can modulated during the time period following memory reactivation, the PCA association is relatively stable after acquisition and is not likely to exhibit post-reactivation lability.

The reason for the difference in vulnerability to post-reactivation manipulations between mCPP and PCA is unclear. However, this difference is representative of the differences in the degree of post-reactivation lability seen in different types of appetitive conditioning. For example, post-reactivation protein synthesis inhibition does not affect the reconsolidation of a basic instrumental conditioning task (Hernandez and Kelley, 2004), but when *zif268* antisense oligodeoxynucleotides were infused into the BLA after re-exposure to a cocaine-associated stimulus, both cue-induced reinstatement of drug-seeking the acquisition of a new conditioned reinforcement response were prevented (Lee et al., 2005; Lee et al., 2006). More study is needed to determine why different types of appetitive conditioning are differentially vulnerable to post-reactivation experimental manipulations. As discussed in Chapter Four, it is possible that strategies based on the post-reactivation lability of memories might prove useful in the treatment of a variety of psychopathologies related to appetitive conditioning, but in order to develop smart clinical strategies, the dynamic properties of appetitive conditioning need to be better understood.

The involvement of the nucleus accumbens in appetitive and aversive Pavlovian conditioning

While neither the NAcc core nor the shell appears to be involved in the acquisition of PCA, experiments from Chapter Four provide evidence that the NAcc core (although not the NAcc shell) is required for acquisition of cued fear conditioning, a form of aversive Pavlovian conditioning. Since the NAcc has classically been thought to be important for behaviors involving reward and motivation (Mogenson et al., 1980; Cardinal et al., 2002b; Balleine, 2005), it is surprising that the NAcc core appears to be involved in the acquisition of aversive, but not appetitive, conditioning.

Not only does the NAcc core seem to be generally involved in the acquisition of cued fear conditioning, but new protein synthesis is required in the core for the acquisition of this behavior (see Chapter Four). This suggests that protein synthesis-dependent plasticity is required in a brain region outside the classical fear circuitry for the acquisition of cued fear conditioning. The most widely held model of the neurocircuitry of cued fear conditioning posits that information is relayed from sensory areas (such as the auditory thalamus) to the BLA; information is hypothesized to flow serially from the BLA to the central nucleus of the amygdala (CeA) and finally to the periacqueductal gray (PAG), a nucleus shown to be required for the freezing response (Fanselow and LeDoux, 1999; LeDoux, 2000, 2003; Kim and Jung, 2006). Protein synthesis has been shown to be necessary within the auditory thalamus, the BLA, and the CeA for the consolidation of auditory fear conditioning (Schafe and LeDoux, 2000; Parsons et al., 2006; Wilensky et al., 2006). However, the results showing that protein synthesis is required in the NAcc core for the acquisition of cued fear conditioning represents the first case where protein

synthesis (and presumably the subsequent plasticity) is required in a brain region outside the amygdala-based fear circuitry, and as discussed in Chapter Four, it would be hard to fit this finding into the classic fear conditioning model of serial processing in the amygdala.

These results could be explained, though, if there was a parallel output circuit underlying cued fear conditioning, and in fact, current neuroanatomy knowledge supports this hypothesis (see Figure 2). The BLA has been shown to project to the NAcc core, and there is also a projection from the NAcc core to the PAG (Usuda et al., 1998; Zahm, 1999, 2000). If this projection is an alternative output pathway to the serial BLA-CeA-PAG pathway, it would explain our results and the results of another recent study investigating the role of the CeA in fear conditioning (Koo et al., 2004).

Conclusions

Like many research projects, the studies contained in this dissertation have raised more questions than they have answered, and there are many possible directions for future studies. Hopefully, future research will be able to fully elucidate the neural circuitry and molecular pathways underlying the consolidation of appetitive Pavlovian conditioning tasks such as mCPP and PCA as well as aversive Pavlovian conditioning tasks such as cued fear conditioning. In addition, future studies could elucidate the circuitry and molecular pathways involved in the reconsolidation of different types of appetitive Pavlovian conditioning and could help us to better understand the dynamic properties of appetitive conditioning in general. A better understanding of the mechanisms underlying the formation and maintenance of these types of associations could potentially lead to

new and more effective treatments for a variety of human psychopathologies, including addiction and compulsive eating disorders.

References

Ammassari-Teule M (2001) Drug addiction and memory systems: how neural stimuli can gain control of behaviour. Functional Neurology 16:227-235.

Balleine BW (2005) Neural bases of food-seeking: affect, arousal and reward in corticostiatolimbic circuits. Physiology and Behavior 86:717-730.

Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecualr mechanims of memory. Neuron 25:151-532.

Brown RW, Bardo MT, Mace DD, Phillips SB, Kraemer P (2000) D-amphetamine facilitation of Morris water task performance is bloced by eticlopride and correclated with increase dopamine synthesis in the prefrontal cortex. Behavioural Brain Research 114:135-143.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002a) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neuroscience and Biobehavioral Reviews 26:321-352.

Cardinal RN, Parkinson JA, Lachenal G, Halkerston KM, Rudarakanchana N, Hall J, Morrison CH, Howes SR, Robbins TW, Everitt BJ (2002b) Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. Behavioral Neuroscience 116:553-567.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci USA 102:6189-6194.

Di Chiano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/Kainate, and dopamine receptors in the nucleus accumbens core in teh acquisition and performance of pavlovian approach behavior. J Neurosci 21:9471-9477.

Doty BA, Doty LA (1966) Facilitative effects of amphetamine on avoidance conditioning in relation to age and problem difficulty. Psychopharmacologia 9:234-241.

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward: the role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 877:412-438.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Fenu S, Di Chiara G (2003) Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D_1 receptors. European Journal of Neuroscience 18:2025-2030.

Hernandez PJ, Kelley AE (2004) Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. Learning and Memory 11:748-754.

Hitchcott PK, Bonardi CMT, Phillips GD (1997a) Enhanced stimulus-reward learning by intra-amygdala administration of a D_3 dopamine receptor agonist. Pyschopharmacology 133:240-248.

Hitchcott PK, Harmer CJ, Phillips GD (1997b) Enhanced acquistion of discriminative approach following intra-amygdala d-amphetamine. Psychopharmacology 132:237-246.

Hsu EH, Schroeder JP, Packard MG (2002) The amygdala mediates memory consolidation for an amphetamine conditioned place prefernce. Behavioural Brain Research 129:93-100.

Janak PH, Martinez JL, Jr. (1992) Cocaine and amphetamine facilitate retention of jump-up responding in rats. Pharmacology, Biochemistry and Behavior 41:837-840.

Kim JJ, Jung MW (2006) Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neuroscience and Biobehavioral Reviews 30:188-202.

Koo JW, Han JS, Kim JJ (2004) Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. J Neurosci 24:7654-7662.

Krivanek JA, McGaugh JL (1969) Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. Agents and Actions 1:36-42.

Kulkarni AS (1968) Facilitation of instrumental avoidance learning by amphetamine: an analysis. Psychopharmacologia 13:418-425.

LeDoux JE (2000) Emotion circuits in the brain. Annual Reviews in Neuroscience 23:155-184.

LeDoux JE (2003) The emotional brain, fear, and the amygdala. Cellular and Molecular Neurobiology 23:727-738.

Lee EH, Ma YL (1995) Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. Brain Research Bulletin 37:411-416.

Lee JLC, Milton AL, Everitt BJ (2006) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. J Neurosci 26:5881-5887.

Lee JLC, Di Chiano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. Neuron 47:795-801.

McGaugh JL (1966) Time-dependent processes in memory storage. Science 153:1352-1258.

Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. J Neurosci 26:3010-3020.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Progress in Neurobiology 14:69-97.

Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. Behavioral Neuroscience 114:42-63.

Parsons RG, Reidner BA, Gafford GM, Helmstetter FJ (2006) The formation of auditory fear memory requires the synthesis of protein and mRNA in the auditory thalamus. Neuroscience 141:1163-1170.

Phillips GD, Setzu E, Hitchcott PK (2003) Facilitation of Appetitive Pavlovian Conditioning by *d*-Ampetamine in the Shell, but Not the Core, of the Nucleus Accumbens. Behavioral Neuroscience 117:675-684.

Robinson MJF, Franklin KBJ (2007) Effects of anisomycin on consolidation and reconsolidation of a morphine-conditioned place preference. Behavioural Brain Research 178:146-153.

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. Journal of Neuroscience 20:1-5.

Schroeder JP, Packard MG (2002) Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. Behavioral Neuroscience 116:922-927.

Setlow B, Holland PC, Gallagher M (2002) Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned response. Behavioral Neuroscience 116:267-275.

Setlow B, Schoenbaum G, Gallagher M (2003) Neural encoding in ventral striatum during olfactory discrimination learning. Neuron 38:625-636.

Soetens E, D'Hooge R, Hueting JE (1993) Amphetamine enhances human-memory consolidation. Neuroscience Letters 161:9-12.

Soetens E, Casaer S, D'Hooge R, Hueting JE (1995) Effect of amphetamine on long-term retention of verbal material. Psychopharmacology 119:115-162.

Strupp BJ, Bunsey M, Levitsky D, Kesler M (1991) Time-dependent effects of post-trial amphetamine treatment in rats: evidence for enhanced storage of representational memory. Behavioral and Neural Biology 56:62-76.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine sutdy. Brain Research 797:73-93.

Valjent E, Corbille A-G, Bertran-Gonzalez J, Herve D, Girault J-A (2006) Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. PNAS 103:2932-2937.

Voorn P, Vanderschuren LJMJ, Groenewegen HJ, Robbins TW, Pennartz CMA (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends in Neurosciences 27:468-474.

Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE (2006) Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. Journal of Neuroscience 26:12387-12396.

Yim AJ, Moraes CRG, Ferreira TL, Oliveira MGM (2006) Protein synthesis inhbition in the basolateral amygdala following retrieval does not impair expression of morphine-associated conditioned place preference. Behavioural Brain Research 171:162-169.

Zahm DS (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Annals of the New York Academy of Sciences 877:113-128.

Zahm DS (2000) An integrative neuroanatomical perspecitve on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neuroscience and Biobehavioral Reviews 24:85-105.

Figure Legends

Figure 1. Neural circuitry supporting the hypothesis that either the basolateral amygdala or the anterior cingulate cortex is the location for the plasticity underlying the CS-US association formed in the PCA task. VTA = ventral tegmental area; Ant. Cing. = anterior cingulate cortex; BLA = basolateral amygdala; NAcc core = nucleus accumbens core; NAcc shell = nucleus accumbens shell

Figure 2. Proposed model for parallel circuitry underlying the acquisition of cued fear conditioning.

*Presumed sites of protein synthesis plasticity based on this dissertation and other work from the LeDoux laboratory (Schafe and LeDoux, 2000; Wilensky et al., 2006). BLA = basolateral amygdala; CeA = central nucleus of the amygdala; NAcc = nucleus accumbens; PAG = periacqueductal gray

Figure 1

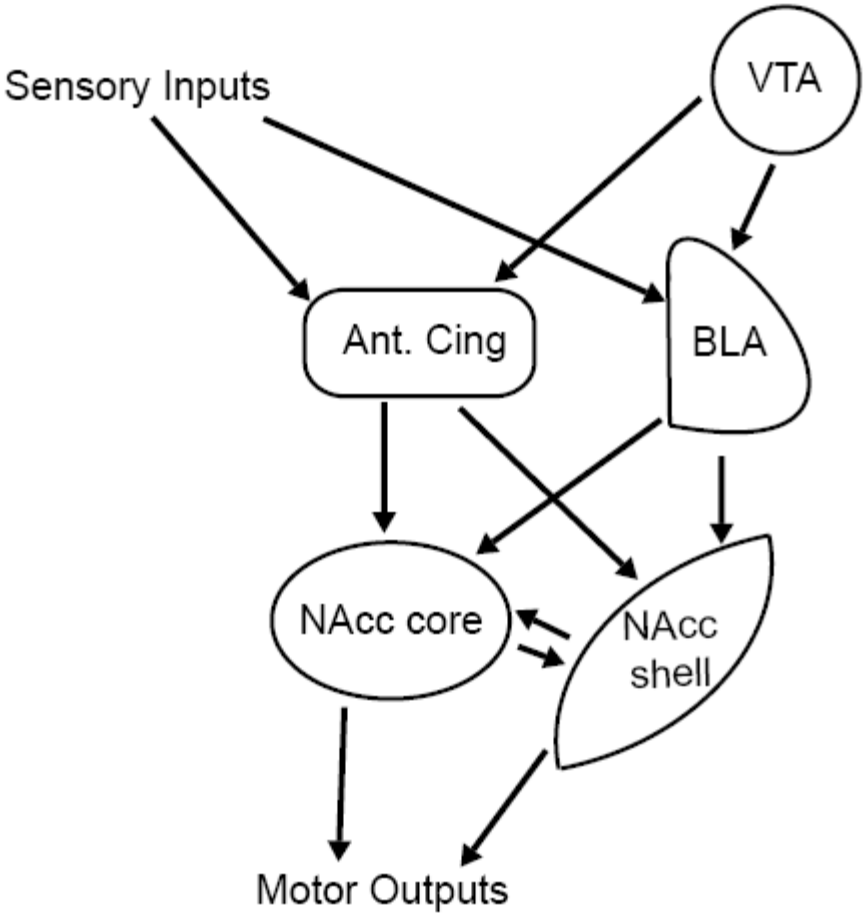
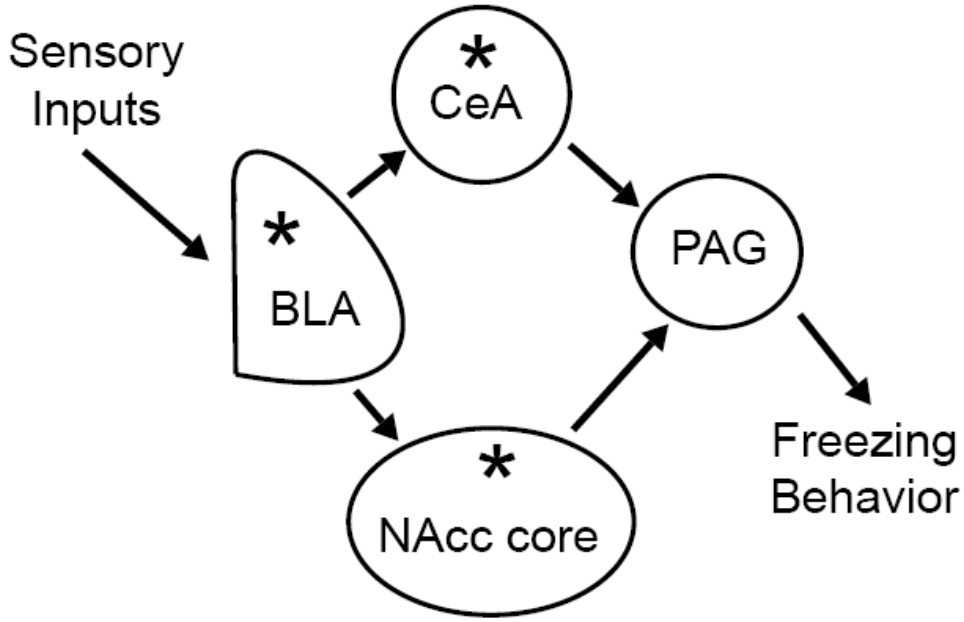

Sensory Inputs
VTA
Ant. Cing
BLA
NAcc core
NAcc shell
Motor Outputs

Figure 2



It is the policy of the University to encourage the distribution of all theses and dissertations. Copies of all UCSF theses and dissertations will be routed to the library via the Graduate Division. The library will make all theses and dissertations accessible to the public and will preserve these to the best of their abilities, in perpetuity.

I hereby grant permission to the Graduate Division of the University of California, San Francisco to release copies of my thesis or dissertation to the Campus Library to provide access and preservation, in whole or in part, in perpetuity.

Author Signature

6/18/2007

Date