UNIVERSITY OF CALIFORNIA, SAN DIEGO

# The Enhancement and Impairment of Learning by Stimulants

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Psychology and Cognitive Science

by

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Professor Stephan G. Anagnostaras, Chair
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Professor Larry Squire
Professor Ben Williams

2010

The Dissertation of Suzanne Courtney Wood is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2010

# DEDICATION

I dedicate this dissertation to my dad, Gordon “Splinter” Wood (1948-2007).

Dad used to maintain that he would never teach any of us kids the card game, Bridge, until we received our advanced degrees. He worried that Bridge was so addictive we would never make it through our respective graduate programs knowing how to play. After enough persuasion, he taught me how to play with the help of his friends, Gary and Deb Miller, in Lehigh, Pennsylvania, on one hot summer afternoon before I started this PhD program.

Dad, I made it!

# EPIGRAPH

"Think for yourself.  Do what you think is right."

Gordon Wood

"Whatever you do, don't go to grad school in Classics."

Jed Parsons, Ph.D., Latin

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Chapter 3, in full, is a reprint of the material as it appears in Amphetamine and extinction of cued fear. *Neuroscience Letters*, 468, 18-22. Carmack, S.A., Wood, S.C., & Anagnostaras, S.G. (2010). The dissertation author was the secondary investigator and author of this paper.

Chapter 4, in full, has been submitted for publication of the material as it may appear in Interdependence of Measures in Pavlovian Conditioned Freezing in *Behavioral Neuroscience*. Wood, S.C. & Anagnostaras, S.G. (2010). The dissertation author was the primary investigator and author of this material.

Chapter 5, in full, is currently being prepared for submission for publication of the material. Wood, S.C., Sage, J.R., Shuman, T., & Anagnostaras, S.G. The dissertation author was the primary investigator and author of this material.

## CURRICULUM VITAE

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## PUBLICATIONS

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ABSTRACT OF THE DISSERTATION

The Enhancement and Impairment of Learning by Stimulants

By

Suzanne Courtney Wood

Doctor of Philosophy in Psychology and Cognitive Science

University of California, San Diego, 2010

Professor Stephan G. Anagnostaras, Chair

Stimulants are prescribed widely to treat a number of disorders, including narcolepsy, shift work sleep disorder, and attention deficit hyperactivity disorder. These prescription stimulants are also commonly, illicitly used for studying and general cognitive enhancement, a trend referred to today as academic doping. Higher doses of stimulants may also be self-administered with a rapid route of administration (e.g., smoking, intravenous injection), leading instead to cognitive impairments and addiction. Few studies have examined the parameters outlining when stimulants switch from being nootropic to harmful. This dissertation proposes that, along with

route of administration, dose is the critical component dictating the cognitive effects of stimulants.

Chapter 1 describes our first study examining the dose-dependent effects of stimulants on fear conditioning, using cocaine. The lowest dose of cocaine enhanced learning, while the highest dose impaired it. Chapter 2 follows with a description of the dose-dependent effects of amphetamine on fear conditioning. Similarly, the lowest doses of amphetamine enhanced learning, while the highest doses led to impairment. Chapter 3 outlines our attempts to extrapolate the amphetamine results to a related form of learning, extinction. We utilized low dose amphetamine to increase extinction learning, but found that no dose facilitated extinction, compared to placebo. Chapter 4 looks further into the structure of fear conditioning, examining the relationships between the most commonly reported training and testing measures. Our analyses demonstrate that post-shock freezing is highly correlated with context fear, confirming that the post-shock measure is better described as a context measure rather than a conditioned response. Our analyses also indicate that tone baseline freezing is not independent from tone test freezing, confounding the most common ways of reporting tone fear. Chapter 5 concludes the dissertation with a broad overview of popular stimulants today, their mechanisms of action, and effects on cognition. A continuum of activation is proposed, in which low doses of stimulants lead to increased concentration and cognitive performance, while high doses lead to cognitive impairments and addiction.

# INTRODUCTION

## The Enhancement and Impairment of Learning by Stimulants

Stimulants are a broad class of drugs used medicinally today for treatment of disorders such as narcolepsy, shift work sleep disorder, and attention deficit hyperactivity disorder (ADHD). These same compounds, however, may also lead to cognitive impairment and addiction. Many theories have been proposed to explain why certain people are more functional when taking stimulants, while others suffer severe impairments and addiction. In the following studies, we propose that, in addition to route of administration, dose of drug is the critical factor in determining cognitive and behavioral effects. Using mice, we examined the effects of different doses of stimulant drugs on learning and memory tasks. We predicted that stimulants administered at low doses would generally enhance learning, while higher doses would lead to learning impairments.

Some of the most publicized stimulants are those closely tied with addiction: amphetamine in its various formulations (e.g., methamphetamine, crystal meth, etc.), and cocaine. While it is well known that there is a host of physical ailments that result from prolonged use of such drugs, deleterious cognitive effects have also been found. Difficulties with attention, social functioning, decision making, episodic memory, processing speed, and motor skills have been frequently documented after prolonged stimulant abuse and addiction (Chung *et al.*, 2007; Henry, Mazur, & Rendell, 2009; Homer *et al.*, 2008; Nordahl, Salo, & Leamon, 2003; Paulus *et al.*, 2002; Scott *et al.*, 2007).

Legal use of stimulants is also quite prevalent, with a long history of use in the military. The unpredictability of war, along with situations such as lengthy air missions, has led to great interest in seeking out alternatives to sleep. During World War II, Benzedrine, a brand name of amphetamine, was examined for its possible benefits to soldiers, in response to reports that other countries were administering the drug to their soldiers (Somerville, 1946). The military continues more modest use today, with care to determine safe and effective guidelines for use of "Go Pills" during lengthy missions. In a study performed on Canadian soldiers, the placebo group dropped in cognitive performance by 30-40% after 24 h without sleep, and 55-65% after 48 h. The soldiers who had periodically received amphetamine declined only by 5-10% after 24 h, and 20-30% after 48 h (Pigeau *et al.*, 1995). Positive effects of dextroamphetamine on performance has led to the conclusion that military personnel may be kept awake for up to 72 hours with its regular administration (Caldwell, 2003).

Stimulants are also often prescribed for a variety of conditions, including narcolepsy and shift work sleep disorder. It is ADHD, however, that has led to the large surge in the number of stimulant prescriptions in recent years. Global prevalence of ADHD has been estimated to be 5.29% of those under the age of 18 (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), with approximately 4.4 million children, or 7.8% of the population, aged 4-17 having a history of ADHD diagnosis in the U.S. (Visser & Lesesne, 2005). Of those children in the U.S., over half (2.5 million) are taking medication for ADHD.

Stimulants remain the primary treatment for ADHD, with studies dating as far back as the 1930s reporting scholastic benefits with stimulant use (Bradley, 1937). Stimulants such as methylphenidate (Concerta, Focalin) and amphetamine (Adderall) have generally been found to be safe and effective for treating ADHD symptoms (Abikoff *et al.*, 2004; Ahmann *et al.*, 2001; Muniz *et al.*, 2008; Spencer *et al.*, 2006). The rate of prescription stimulant use in children aged 18 or younger jumped from 0.6% to 2.4% between 1987 and 1996 (Olfson, Marcus, Weissman, & Jensen, 2002), but the rate remained stable between 1997 and 2002, with a nonsignificant increase from 2.7% to 2.9% of children 19 years old and younger using stimulants (Zuvekas, Vitiello, & Norquist, 2006). Research on non-stimulant compounds for ADHD treatment is ongoing, but generally has found these treatments to be less effective than stimulants, as seen with atomoxetine (Faraone, Wigal, & Hodgkins, 2007), or to have equally disruptive side effects, such as sedation, as seen with guanfacine (Biederman *et al.*, 2008; Faraone & Glatt, 2010).

While those with ADHD are helped by use of stimulants, a portion of the general population is also partaking of these drugs for an extra edge in scholastic performance, commonly referred to as academic doping. While the debate over the use of cognitive enhancers is typically considered a new discussion, reflecting the emergence of our fast-paced culture, evidence for academic doping can be found in literature dating back to the 1940s (Nathanson, 1942). In more recent years, studies have been conducted to attempt to grasp how prevalent academic doping is among young adults, specifically college students. A nationwide survey of over ten thousand

students from 199 U.S. colleges and universities estimated the lifetime prevalence of nonmedical stimulant use to be 6.9%, with 4.1% using within the previous month (McCabe, Knight, Teter, & Wechsler, 2005). A survey of 3,401 university students found an estimated 13.3% had illicitly used prescription stimulants at least once in their lives (Arria *et al.*, 2008). A recent review found a total of 21 studies on the nonmedical use of prescription stimulants, including 113,145 participants, with rates of stimulant use ranging from 5% to 35% within the preceding year (Wilens *et al.*, 2008). While these estimates vary to some degree, is clear that academic doping is widespread.

Altogether, there is much evidence for both cognitive impairment as well as enhancement with stimulant use. Many explanations have been popularized over the years to account for these findings. One common misperception is that those with ADHD respond differently to stimulants than those without ADHD. While more recent imaging studies are beginning to find neurological differences in responses to stimulants between those with ADHD and without (Vaidya *et al.*, 1998), few studies have shown large behavioral differences to the extent commonly misperceived by the public. In particular, studies have not shown that those with ADHD are dramatically calmed by a stimulant, while those unaffected become hyper and jittery by that same stimulant. While decades of research have supported this disclaimer (Agay, Yechiam, Carmel, & Levkovitz, 2010; Rapoport, Buchsbaum, & Weingartner, 1980; Rapoport *et al.*, 1978; Sahakian & Morein-Zamir, 2007), as has the growing industry of academic doping described above, a quick online search shows the rumor being perpetuated on a

plethora of non-scientific blogs and forums. Similar logic applies to another common misperception that children, alone, can develop ADHD, and that they are calmed by stimulants, while adults always become more hyperactive. It is indisputable today that adults can develop ADHD, with current research focusing on what special considerations should be taken in diagnosing and treating adult ADHD (Davidson, 2008). Finally, the specific drug, alone, does not seem to dictate the benefits or detriments experienced after use. For example, while methylphenidate in pill form is considered generally safer than cocaine and is commonly prescribed, injected liquid methylphenidate is said to feel much "like cocaine" to addicts, according to Dr. Nora Volkow (Vastag, 2001). Methylphenidate was previously considered a mild stimulant, however, its actions at the level of the synapse have been demonstrated to be similar to that of cocaine, leading to a large increase in extracellular dopamine (Volkow *et al.*, 2001). This is an example of a drug typically considered "safe", but having the same potential for addiction at certain doses, and via certain routes of administration.

### *Pavlovian Fear Conditioning and Stimulants*

The studies described here evaluate dose as the critical determinant of the cognitive effects of stimulants. For the first three studies, we utilized Pavlovian fear conditioning as a model of memory in mice. Fear conditioning has rapidly become a modal model of memory, commonly used in genetic and pharmacological studies due to its convenience, efficiency, and reproducibility.

Pavlovian fear conditioning consists of a brief training session, during which one or several repetitions of a stimulus are presented (usually a tone; conditioned stimulus, CS), each co-terminating with an aversive stimulus (usually a mild footshock; unconditioned stimulus, US). From this relatively brief training session, the subject forms long-lasting memories relating the shock not only to the tone (cued or tone fear), but also to the context in which the training took place (contextual fear). Fear memory is typically quantified by measuring freezing (conditioned response, CR), a species-specific behavior defined as the absence of movement aside from respiration (Blanchard & Blanchard, 1969; Fanselow, 1980). Both forms of memory are dependent upon the amygdala, while contextual fear memory also relies upon the hippocampus in a time-dependent manner (Anagnostaras, Maren, & Fanselow, 1999). Due to its dependence on the hippocampus, contextual fear has become a leading model of declarative memory. In this way, fear conditioning provides a means of quickly testing both declarative, as well as non-declarative memory.

In examining the effects of stimulants on Pavlovian fear conditioning, we hypothesized that high doses of stimulants would impair learning, while increasing locomotor activity, and that low doses of stimulants would enhance learning, while decreasing locomotor activity. Presented in the first two chapters of this dissertation are studies examining cocaine and amphetamine, with cocaine generally considered to have little cognitive enhancing effects and be highly addictive, and amphetamine being commonly prescribed today. All mice were injected with placebo or drug 15

min prior to a testing session that consisted of three tone-shock pairings. Mice were tested, off-drug, 24 h later for context fear, and 48 h after training for tone fear.

In Chapter 1, we examined the effects of a range of doses of cocaine on Pavlovian fear conditioning. An acute dose of cocaine was administered (i.p.) 15 min prior to training. Both context and tone fear testing was conducted off-drug. All doses of cocaine led to an increase in activity, compared to placebo, while the animals were on drug. Interestingly, in examining freezing behavior immediately following the tone-shock pairings, freezing was increased in those animals administered the lowest dose of cocaine (0.1 mg/kg), compared to placebo. This effect persisted 24 h later, with the context test, as well as 48 h later, during the tone test. The group that had been administered 0.1 mg/kg cocaine prior to training continued to show a stronger fear memory for both the context and tone when tested off-drug.

The effects of amphetamine on Pavlovian fear conditioning were examined in Chapter 2. Increased locomotor activity was found only in the mice administered the highest doses of amphetamine (4 and 8 mg/kg, i.p.), with the low and moderate doses inducing no hyperactivity (0.005, 0.025, 0.05, 0.5, 1, and 2 mg/kg). The mice on the lowest doses of amphetamine (0.005, 0.025, and 0.05 mg/kg) displayed increased freezing, compared to controls, immediately following the tone-shock pairings. This increased freezing did not generalize to the context test performed the following day. However, the same low dose groups showed increased freezing, compared to placebo, during the tone test.

Considering that low dose amphetamine helped in the acquisition of cued fear conditioning, Chapter 3 examines if it could likewise assist in the extinction of fear memories, also generally considered to be a form of learning (Myers & Davis, 2007). Extinction therapy is commonly used in the treatment of anxiety disorders such as phobias or post-traumatic stress disorder. Recent research has focused on pharmacological compounds to help strengthen this learning, thereby enhancing the outcome prospects for those undergoing treatment (Davis, Ressler, Rothbaum, & Richardson, 2006; Mathew, Price, & Charney, 2008; Powers, Smits, Otto, Sanders, & Emmelkamp, 2009). To examine whether amphetamine could enhance extinction, we presented mice with 9 tone-shock pairings during training, off-drug. Twenty-four hours later, we administered 0.005 mg/kg amphetamine, 0.05 mg/kg amphetamine, or saline 15 min before the first extinction session. Five more days of extinction paired with drug followed, for a total of 6 extinction sessions. Neither dose of amphetamine enhanced extinction, compared to saline, indicating amphetamine may not be useful for extinction training.

*Interdependence of Fear Conditioning Measures*

Pavlovian fear conditioning is a popular model of human declarative memory, yet the relationships between its separate dependent measures are not well understood. Chapter 4 examines each commonly reported measure of fear conditioning and the interdependence between those measures. Post-shock fear, or freezing that is displayed while the animal is still in the conditioning context after the tone-shock

pairings have completed, is strongly correlated with contextual fear measured 24 h later. While contextual and tone fear are considered dissociable measures of declarative and nondeclarative memory, respectively, we found the two measures to also be correlated. Finally, tone baseline freezing, or the period during the tone test preceding the presentation of the tone, correlated with tone freezing. This finding throws into question the typical methods used to account for baseline freezing (e.g., subtraction). A recent publication proposes a new fear conditioning protocol to help alleviate this confound and strengthen the validity of the tone fear measure (Jacobs, Cushman, & Fanselow, 2010).

*Continuum of Stimulant Activation*

Chapter 5 is a review of the stimulant literature, emphasizing dose as the critical determinant of a stimulant's behavioral and cognitive effects. We first discuss the basic mechanisms and cognitive effects of the popular psychostimulants, cocaine, amphetamine, methylphenidate, modafinil, and caffeine. In comparing the differences in effects of low and high doses of the same drugs, we propose a continuum of activation, in which low doses of stimulants lead to cognitive enhancement and high doses of stimulants lead to cognitive deficits and addiction. This continuum parallels the current neurobiological model of attention deficit hyperactivity disorder (ADHD), which focuses on catecholamine levels. This ADHD model posits that symptoms are evident in the bottom, left portion of an inverted U-shaped curve of catecholamine level in the prefrontal cortex (Arnsten, 2009). Peak cognitive performance is found

with a moderate amount of catecholamines present, while high levels are evidenced by stress and, again, poor performance.

Chapter 1

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Research report

# Cocaine and Pavlovian fear conditioning: Dose–effect analysis

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**Abstract**

Emerging evidence suggests that cocaine and other drugs of abuse can interfere with many aspects of cognitive functioning. The authors examined the effects of 0.1–15 mg/kg of cocaine on Pavlovian contextual and cued fear conditioning in mice. As expected, pre-training cocaine dose-dependently produced hyperactivity and disrupted freezing. Surprisingly, when the mice were tested off-drug later, the group pre-treated with a moderate dose of cocaine (15 mg/kg) displayed significantly less contextual and cued memory, compared to saline control animals. Conversely, mice pre-treated with a very low dose of cocaine (0.1 mg/kg) showed significantly enhanced fear memory for both context and tone, compared to controls. These results were not due to cocaine's anesthetic effects, as shock reactivity was unaffected by cocaine. The data suggest that despite cocaine's reputation as a performance-enhancing and anxiogenic drug, this effect is seen only at very low doses, whereas a moderate dose disrupts hippocampus and amygdala-dependent fear conditioning.


*Keywords:* Hippocampus; Amygdala; Freezing; Memory

A growing body of evidence supports the view that drugs of abuse interfere with many aspects of cognitive functioning. For example, cocaine use has been linked to deficits in such cognitive areas as attention, cognitive flexibility, and short-term and working memory [34,43]. However, these findings in humans are controversial as cocaine use is naturally confounded with many other variables [e.g. 23,27,31]. Some studies, in fact, provide evidence for enhancement of certain cognitive abilities in cocaine users [e.g. 27,50]. Relatively few studies in animals have examined the effects of cocaine on learning outside of the realm of addiction (e.g. self-administration, place preference, sensitization). Indeed, several studies have focused on the mechanisms underlying addiction, including structural plasticity [see 41 for review], but few have examined the acute, behavioral effects of cocaine in rodents on simple learning and memory paradigms. We believe that most researchers implicitly assume, as we once did, that cocaine, as a psychostimulant, would naturally enhance learning and memory. However, evidence now exists linking cocaine use with specific cognitive deficits [5,25,34,43] as well as with general problems such as unemployment [35]. Moreover, a view has emerged that addictive drugs such as cocaine may work by taking control of critical reinforcement-related learning and memory circuits in the brain [28]. If cocaine modulates such circuits, then it may interfere with learning and memory. In this experiment, we examined the acute effects of cocaine on an aversively motivated Pavlovian fear conditioning task in mice.

In Pavlovian fear conditioning, an aversive, fear-producing unconditioned stimulus (US) is paired with an initially neutral conditioned stimulus (CS). The US elicits an unconditioned fear response (UR); following training, the CS alone elicits a conditioned fear response (CR). The environmental chamber where conditioning takes place may serve as a CS, a phenomenon known as context conditioning. A common fear conditioning paradigm is pairing a tone (CS) in a specific environmental context (CS) with a footshock (US). Although subjects manifest a number of physiological and behavioral changes after training, a commonly measured fear response is freezing [18]. Because Pavlovian fear conditioning can be monitored particularly efficiently in mice, it has become a popular means of assessing learning and memory in molecular studies [2].

The neuroanatomy of Pavlovian fear conditioning has been thoroughly investigated in recent years. The hippocampus is necessary for forming and temporarily storing memory of the context, while the amygdala is necessary for memory of both

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context and tone fear, and perhaps memory of the shock as well [1,3,19,22,38,40]. Involvement of both the hippocampus [8,36,53,54] and the amygdala [9,16,21,37] has been implicated in cocaine use and addiction. For example, studies by Thompson et al. [44–46] have shown that cocaine exposure modulates long-term potentiation in the CA1 region of the hippocampus. However, the cognitive and behavioral effects of acute cocaine administration on Pavlovian fear conditioning have yet to be examined.

In the current study, mice were injected with cocaine prior to placement in the conditioning chambers. Following training, subjects were tested for both context and tone memories, off-drug. By one view, acute cocaine administration should potentiate fear conditioning, as cocaine is a nootropic psychostimulant [16] and an anxiogenic [4,42]. Studies have shown that cocaine can inhibit the extinction of a fear-potentiated startle response when paired repeatedly with a nonreinforced CS, suggesting that cocaine can intensify the anxiety-producing qualities of a CS [12]. Increased overall arousal or anxiety could enhance the learning of an anxiety-producing stimulus. Alternatively, cocaine could impair fear conditioning, as it disrupts not only the normal activation of the amygdala and hippocampus, but also areas in the prefrontal cortex related to attention [32]. According to this view, subjects on cocaine may not retain memory of context and tone cues because they would be unable to sufficiently attend to them during training. We were interested in characterizing the effects of cocaine on fear conditioning both with doses similar to those taken by human addicts, as well as lower doses, which we believed more likely to enhance learning. A moderate dose of cocaine produced a remarkable impairment in learning and memory, while a very low dose of cocaine enhanced memory. Several interpretations of these findings are presented.

## 1. Methods

### 1.1. Subjects

Sixty-four C57B6/J (B6; Jackson Laboratory, West Sacramento, CA) inbred mice were used. Mice were weaned at 3 weeks of age and were group housed (two to five mice per cage) with continuous access to food and water. The vivarium was maintained on a 14:10 light/dark schedule, and all testing was performed during the light phase of the cycle. Mice were at least 8 weeks old before testing. Approximately equal numbers of male and female mice were used. All animal care and testing procedures were approved by the UCSD IACUC and were in accordance with the NIH Principles of Laboratory Animal Care.

### 1.2. Apparatus

#### 1.2.1. Conditioning context

Three to four mice were tested concurrently, in individual conditioning chambers housed in a windowless room. Each chamber (32 cm wide, 25 cm high, 25 cm deep) was equipped with a speaker in the side wall and a stainless steel grid floor (36 rods, each rod 2-mm diameter, 8-mm center to center; the front wall was clear acrylic, the sidewalls were white acrylic; Med-Associates Inc., St. Albans, VT). A stainless steel drop-pan, scented with 7% isopropyl alcohol to provide a background odor, was located beneath each chamber. Between tests, the conditioning contexts were cleaned with 7% isopropyl alcohol solution. Background noise (65-dB) was provided by a HEPA air cleaner and white light was provided by two 100 W bulbs. The mice were continually observed by a wall-mounted color video camera which was connected to a computer and video equipment in an adjacent room. Each chamber was connected to a solid-state scrambler, providing AC constant current shock, and an audio stimulus generator located in an adjacent room, controlled via an interface connected to a Windows computer running Med-PC (Med-Associates Inc., St Albans, VT). Automated assessment of freezing and activity was provided by custom designed software adapted from NIH Image running on an Apple Macintosh G4 [automated algorithm validated elsewhere, 2].

#### 1.2.2. Alternate context

The alternate context for testing tone fear was located in a separate room and differed from the training context along several dimensions. Multiple (three to four) mice were tested concurrently, in individual boxes measuring 30 cm wide, 25 cm high, 24 cm deep, and equipped with a speaker in the side walls. The ceiling, floor, and three interior walls of the chamber were white, while a clear Plexiglas front wall allowed the mice to be continually observed. To create a distinct space, a white plastic, triangular tent was placed inside each box, with each side of the triangle measuring 23 cm. Between tests, the chambers were cleaned and scented with a 5% white vinegar solution. The room was lit with dim red light and an infrared video camera, connected to the Macintosh G4 described above, was used to score freezing.

### 1.3. Behavior measurement

In order to measure the effect of cocaine on exploratory locomotor activity, baseline activity during training was assessed by counting the number of cross-overs each subject performed [e.g. 38]. A single cross-over was defined as the movement of a subject's entire body from one half of the box to the other. Videotapes of the conditioning sessions were observed using a standard VCR and monitor, and the number of cross-overs was counted during the first 2 min prior to the first tone–shock pairing on the training day.

In addition, to ensure that cocaine did not disrupt shock reactivity, mouse activity burst displayed during the 2 s of shock exposure (Unconditioned Response to shock), as well as activity during the 2 s leading up to the shock were measured as velocity (cm/s) [2]. Full-screen video of this time period was digitized at 10 Hz using NIH Image. *X–Y* coordinates were obtained for each frame for each mouse using the wand auto-measure tool; these coordinates were imported into Microsoft Excel. Distance traveled (measured in pixels) between successive frames was computed using the distance formula $[\sqrt{((x_n - x_{n+1})^2 + (y_n - y_{n+1})^2)}]$; these values were converted into real distance in centimeters using known landmark distances in the video frame. Distance was then converted into velocity (cm/s) by dividing by time.

### 1.4. Drugs

Drugs were administered intraperitoneally (i.p.) in a volume of 5–10 ml/kg. Cocaine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% physiological saline. Cocaine injections (salt weight: 15, 5, 1, or 0.1 mg/kg, i.p.) were given 15 min before training. These doses were selected to form a comprehensive dose–effect curve; although 15 mg/kg is usually regarded as a moderate dose, we found an unacceptable lethality rate (about one-third) at the next highest appropriate dose (30 mg/kg), in this strain of mice, whereas lower doses did not produce any lethality.

## 2. Experimental procedures

### 2.1. Conditioning

Mice were injected with either saline or cocaine 15 min prior to training. Training consisted of a 2 min baseline period, followed by a 30 s tone (28 kHz, 85 dB, A Scale), with a shock (0.75 mA) administered during the last 2 s of the tone. Two more tone–shock pairings followed, separated by 30 s each. Immediate post-shock freezing was measured for another 5 min. Thus, mice were inside the fear conditioning chambers for a total of 10 min, then returned to their home cages.

### 2.2. Testing

Mice were returned to the conditioning context without drug 24 h later and freezing was scored for 5 min. Mice were subsequently placed in the tone test context 48 h later. Tone testing consisted of a 2 min baseline, followed by a

continuous 3 min tone identical to the training tone. Freezing was scored for the entire 5 min period.

## 3. Results

### 3.1. Generalized activity

Cocaine produced an increase in cage cross-overs during the 2 min baseline period on the training day (Fig. 1A). A univariate analysis of variance (ANOVA) confirmed group differences [$F(4, 59) = 7.1$, $p = 0.0001$]. Compared to saline control animals, all doses of cocaine produced significant hyperactivity [0.1 mg/kg, $F(1, 24) = 12.2$, $p < 0.01$; 1 mg/kg, $F(1, 24) = 12.5$, $p < 0.01$; 5 mg/kg, $F(1, 24) = 14.0$, $p < 0.01$; 15 mg/kg, $F(1, 26) = 17.0$, $p < 0.001$], suggesting it is a psychomotor stimulant even at the lowest doses.

### 3.2. Activity burst velocity

Cocaine produced an increase in baseline velocity in only the animals receiving 15 mg/kg in this very brief sample of velocity, compared to control animals [$F(1, 26) = 6.97$, $p < 0.05$; all others, n.s.; Fig. 1B]. Cocaine had no apparent effect on shock reactivity at any dose, with no difference between doses in activity burst velocity during shock [$F(4, 59) = 0.8$, $p > 0.5$]. The shock produced a large increase in velocity compared to baseline in all groups [$F(4, 59) = 428.0$, $p < 0.0001$].

### 3.3. Training and post-shock freezing

Fig. 2A depicts the acquisition of freezing during training. The first 2 min period is a baseline measurement, and is followed by three tone–shock pairings spread over 3 min. Saline control subjects exhibited a higher level of freezing averaged over the 5 min training session compared with those receiving 1, 5, or 15 mg/kg, but did not differ from those receiving 0.1 mg/kg [0.1 mg/kg, $F(1, 24) = 2.92$, $p = 0.10$; 1 mg/kg, $F(1, 24) = 5.57$, $p < 0.05$; 5 mg/kg, $F(1, 24) = 25.0$, $p < 0.0001$; 15 mg/kg, $F(1,$

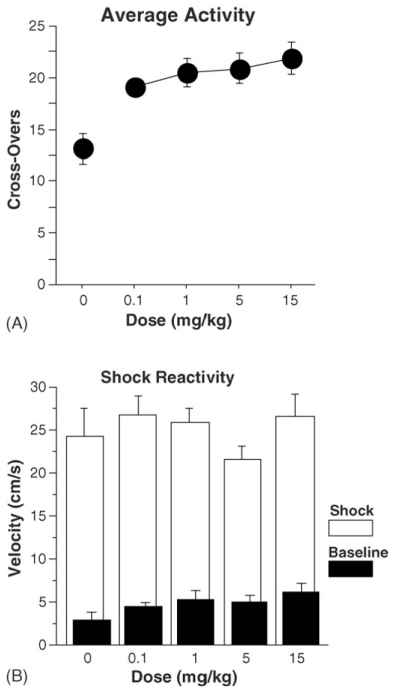


Fig. 1. (A) Average activity: cage cross-overs were counted during the 2 min baseline period before the onset of the tone and shock on training day. Subjects receiving 0.1, 1, 5, or 15 mg/kg cocaine, i.p., 15 min prior to training showed hyperactivity compared to saline control subjects. (B) Shock reactivity: velocity was measured during the 2 s prior to the first shock (baseline) and during the 2 s of the first shock (shock). Only subjects receiving 15 mg/kg showed an increase in velocity during baseline, compared to controls. All groups showed a marked increase in velocity from baseline to shock. The velocity during shock did not differ between groups, showing no dose-dependence in Unconditioned Response.

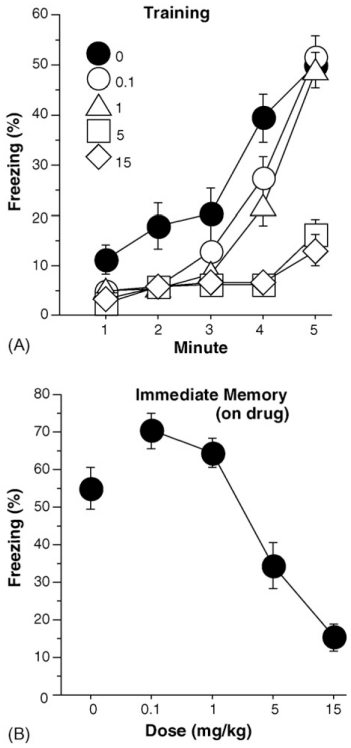


Fig. 2. (A) Training: freezing was measured continuously throughout the initial, 2 min baseline period, as well as the 3 min period encompassing three tone–shock pairings. Saline control subjects displayed more freezing than most other groups (1, 5, and 15 mg/kg cocaine). Subjects receiving 5 or 15 mg/kg cocaine displayed much lower levels of freezing during the three tone–shock pairings. (B) Immediate shock memory: freezing was measured for 5 min immediately following training, on drug. Dose-dependent effects are evident, with animals on 0.1 mg/kg cocaine displaying more freezing than saline control animals. Animals injected with 5 or 15 mg/kg cocaine froze significantly less than controls.

26) = 30.2, $p < 0.0001$]. Subjects receiving 5 or 15 mg/kg of cocaine displayed much less freezing than all other groups during the tone–shock pairings ($F$ values > 25, $p$ values < 0.0001). Fig. 2B depicts the average level of freezing during an immediate memory test that consisted of leaving the mice in the chambers for an additional 5 min, on-drug. Dose-dependent effects on post-shock freezing are evident [ANOVA, $F(4, 59) = 22.1$, $p < 0.0001$], with 0.1 mg/kg producing a nearly significant elevation in freezing, despite producing hyperactivity, compared with controls [$F(1, 24) = 4.1$, $p = 0.05$]. There was no difference between saline and 1.0 mg/kg cocaine groups, while there was a large deficit in freezing in both the 5 mg/kg as well as 15 mg/kg groups, compared with saline controls ($F$ values > 5, $p$ values < 0.05). Fifteen milligrams per kilogram produced a larger deficit than 5 mg/kg [$F(1, 24) = 8.0$, $p < 0.01$].

This post-shock freezing measurement also, in part, addresses the issue of state-dependency in learning [e.g. 39]. The state-dependency view would predict that the decrement in freezing while on-drug, during training, would be less than that seen off-drug, during testing. However, the freezing decrement seen on-drug is greater than that seen off-drug.

### 3.4. Context fear

One day after training, mice were returned to the context in which they had been trained, off-drug. A dose-dependent effect of cocaine can be seen in the average rate of freezing over the 5 min context test (Fig. 3A) [ANOVA, $F(4, 59) = 7.8$, $p < 0.0001$]. Subjects pre-treated with 0.1 mg/kg during training displayed enhanced freezing, compared to saline controls, during the context test, off-drug [$F(1, 24) = 4.71$, $p < 0.05$]. Mice pre-treated with 15 mg/kg showed a significant decrement in freezing [$F(1, 26) = 8.88$, $p < 0.01$], while those pre-treated with 1 or 5 mg/kg were not significantly different than saline controls ($F$ values < 1). In contrast to the immediate memory test, it is unlikely that response competition was a factor, since mice were tested off-drug, suggesting that cocaine given during training somehow interfered with the memory for fear conditioning. Another, although unlikely (see below) explanation is that conditioned hyperactivity, by virtue of drug-environment conditioning, produced an increase in activity in cocaine pre-treated mice that disrupted freezing.

### 3.5. Tone fear

Two days after training, mice were administered the tone test in a novel context, off-drug. A dose-dependent response to the 3 min presentation of the tone is shown in Fig. 3B; there were significant differences [$F(4, 59) = 3.8$, $p < 0.01$]. Mice administered 0.1 mg/kg cocaine, pre-training, displayed an increase in freezing during the tone test compared with saline controls [$F(1, 24) = 4.45$, $p < 0.05$]. Mice pre-treated with either 1 or 5 mg/kg displayed no difference in performance compared with controls ($F$ values < 0.5), while mice administered 15 mg/kg showed a decrement in performance [$F(1, 26) = 4.50$, $p < 0.05$]. These findings do not support the view that the cocaine pre-treated mice exhibited deficits in freezing due to conditioned hyperactivity,

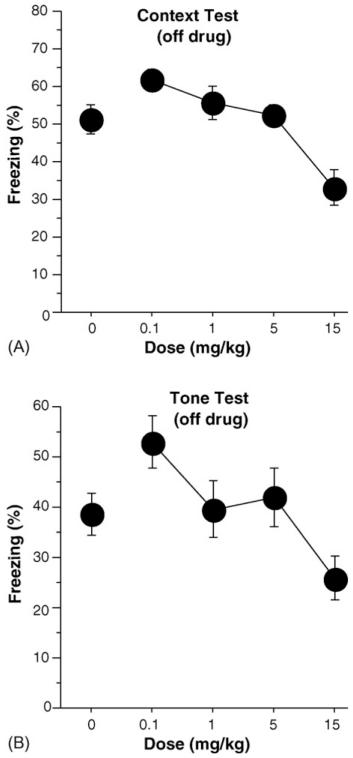


Fig. 3. (A) Context Test: 24 h after conditioning, subjects were returned to the training context, off-drug. Freezing was measured for 5 min. Subjects which had received 0.1 mg/kg of cocaine, pre-training, displayed an increase in freezing, compared to saline control subjects. Subjects pre-treated with 15 mg/kg displayed a deficit in freezing. (B) Tone Test: 2 days after training, subjects were introduced to a new context with no additional stimuli for 2 min, then presented with the tone from training for 3 min. Subjects given 0.1 mg/kg before training showed an increase in freezing, off-drug, compared to saline controls, while those given 15 mg/kg showed a decrease in freezing.

since cocaine was never paired with this context. In addition, there was no difference in freezing between groups during the 2 min baseline period [$F(4, 59) = 0.9$, $p > 0.45$; data not shown].

Overall, mice exhibited an enhancement of context and tone memory tested off-drug when given 0.1 mg/kg, and a deficit in memory when given 15 mg/kg of cocaine. Five milligrams per kilogram was also able to disrupt immediate memory, while the mice were still on the drug.

Finally, doses of 0.1–15 mg/kg all produced hyperactivity, suggesting these are all still doses at which cocaine is acting as a psychostimulant.

## 4. Discussion

Administration of a very low dose of cocaine before fear conditioning enhanced freezing during both context and tone testing when subjects were off-drug, while a moderate dose disrupted

memory during both tests. These results were surprising, as there are many reasons to expect a moderate dose of cocaine should potentiate fear conditioning. For example, cocaine is thought of as an anxiogenic, amplifying many anxiety and defensive behaviors in rodents [e.g. 4,42,55], and thereby would be predicted to increase the response to fear conditioning. Cocaine has also been shown to increase the fear-potentiated startle response in rats, as well as inhibit extinction to a CS [10,12,52]. In addition, psychostimulants, such as caffeine, are usually thought of as nootropics [e.g. 15,26,49]. This folklore is exemplified by the advertisement campaign of the original Coca-Cola that contained cocaine, declaring it "the brain tonic and intellectual soda-fountain beverage" [51]. However, despite its anxiogenic and nootropic effects, only a very low dose of cocaine increased performance on fear conditioning, while a moderate dose disrupted it.

As fear conditioning is known to require activation of both the hippocampus and amygdala [1,3,19,20,38], one explanation for the results is that cocaine altered the regular functioning of the amygdala, the hippocampus, or both. In another study on fear conditioning, Corodimas et al. [17] discovered the acute administration of caffeine, pre-training, produced a selective deficit in context conditioning only, suggesting a disruption of hippocampal function. Repeated exposure to cocaine can enhance long-term potentiation (LTP) in the CA1 region of the hippocampus [44]. However, this effect may be reversed at high concentrations of cocaine, or after long periods of withdrawal [45,46], and has not been shown with an acute, in vivo exposure to cocaine. It is possible, though, that this enhanced LTP could account for the better performance during testing of the subjects which had received 0.1 mg/kg cocaine before training. Other studies on both acute and frequent cocaine intake have revealed the disruption of amygdala-dependent learning, as well [29,48]. The present effects on both context and tone conditioning suggest that both hippocampus and amygdala-dependent learning can be disrupted even by acute, moderate cocaine administration, while a very low dose can enhance this learning.

One possibility is that cocaine interfered with motivational components of fear conditioning. Cocaine is a powerful local anesthetic, and it is possible that a 15 mg/kg, i.p. injection could have extended some anesthesia to the paws, reducing the painfulness of the footshock. However, measurement of the mouse activity burst UR suggests that footshock reactivity was equivalent in cocaine and saline-treated mice. Alternatively, the cocaine could have induced a pleasurable hedonic state, in which the animals on cocaine may have perceived the shock or fear conditioning experience as less aversive. This possibility would be difficult to explore within the current experiment, but could suggest that all drugs which induce a positive hedonic state may interfere with aversive conditioning. This seems unlikely, as there is evidence that cocaine, despite being thought of as producing a positive hedonic state, amplifies other anxiety and defensive behaviors [4]. Finally, there is also evidence from studies in monkeys that cocaine can disrupt an appetitively motivated working memory task as well [29].

Another possibility is that cocaine directly disrupted conditioning by interfering with learning and memory circuits. Human chronic cocaine users have been shown to have deficits in measures of attention [see 34 for review], while dysfunction in prefrontal cortex activity has been implicated for attentional deficits in several studies of drug abusers [6,7,24,25,30,32,47]. Thus, it is possible that cocaine directly disrupted the circuits required for learning and memory in fear conditioning.

A possible confound in the present study is that we were unable to directly control for state-dependent learning. By this argument, on-drug testing may allow better recall of the memory of the context, tone and shock pairings, after on-drug training. Because the subjects were tested off-drug, one can argue that they were at a disadvantage, compared to controls, because of their different drug state during training and testing sessions. However, subjects which had received 0.1 mg/kg cocaine before training continued to show higher levels of freezing than controls when tested off-drug. State-dependent learning would predict quite the opposite result. Moreover, when the immediate memory test was used to control for state-dependency, mice were actually more sensitive to the disruptive effects of cocaine than when tested off-drug. These findings agree with previous research addressing similar issues. Studies examining the effects of repeated cocaine administration on fear conditioning, using the fear-potentiated startle paradigm, found that state-dependent learning did not satisfactorily account for increased startle amplitudes, after extinction [52]. Overall, there is little evidence that cocaine or other stimulants produce heavily state-dependent learning in humans or in other animals [39].

Although it is likely that hyperactivity directly attributable to the drug is responsible for freezing deficits seen during training, hyperactivity seems an unlikely explanation for deficits in context and tone memory when mice were tested off-drug. Conditioned hyperactivity via drug-environment conditioning could theoretically play a role in the apparent context and tone memory deficit seen in animals given 15 mg/kg cocaine. However, it is unlikely that much conditioned hyperactivity would be produced by a single drug exposure. Moreover, mice also exhibited deficits in tone testing, which was tested in a context never paired with cocaine, suggesting that conditioned hyperactivity played little role in the freezing deficits seen on testing. Finally, subjects which had been administered 0.1 mg/kg cocaine before training exhibited an increased level of activity during the 2 min baseline period, but exhibited increased freezing during both the context and tone tests, off-drug. Taken together this suggests that conditioned hyperactivity is not directly responsible for the memory deficits seen in context and tone conditioning.

An emerging view of addiction states that drugs of abuse hijack existing learning and memory systems normally involved in conditioned reinforcement [28]. If this is true, these neural systems may be unable to properly participate in the forms of memory they normally subserve. Given that cocaine alters the normal functioning of ventral tegmental area (VTA)–nucleus accumbens (NAcc) connectivity, it may disrupt the functional reward system of the brain. Human studies have implicated areas of the amygdala in this disruption, as well, correlating amygdala activation with cocaine craving [e.g. 13,14]. If this is the case, then areas of the brain normally used for encoding the context

and tone information during fear conditioning would be disrupted and unable to encode new information.

In addition, VTA dopamine neurons have been implicated in both the behavioral expression of a fear response as well as memory. For example, lesions to the VTA were shown to inhibit fear-potentiated startle in rats [11]. Also, cocaine administered repeatedly over 5–7 days, but not acutely on a single day, facilitated LTP induction in VTA dopamine neurons [33]. Together, these results suggest that repeated cocaine administration could enhance memory of a fear response via VTA dopamine neurons. These results also predict that an acute, moderate dose of cocaine, as used in the present study, would not be expected to enhance memory encoded via these neurons.

In the present study we chose to focus on a range of cocaine doses, from a low dose which is unlikely self-administered by drug users, to a moderate dose thought to be typical of drug users. Within that range, we found differing results. Future studies will use other psychostimulants [in preliminary studies with modafinil (Provigil®) we have found equivalent results]. We hope to determine whether a moderate dose of cocaine is unique in its detrimental effects on learning, or whether the acute administration of other stimulants may produce other previously unknown learning deficits, as well. Likewise, we will study if low doses of other psychostimulants also enhance learning.

These surprising results indicate that even an acute administration of a moderate dose of cocaine can be highly detrimental to learning and cognition. In particular, both hippocampus-dependent context memory and amygdala-dependent tone memory of Pavlovian conditioning were markedly disrupted by a moderate pre-training dose of cocaine. Taken together, these data suggest that many unexplored cognitive deficits may be produced by moderate doses of psychostimulant drugs that are normally thought of as performance-enhancing. Specifically, these renowned performance-enhancing effects may only be seen at doses realistically too low to be administered by drug users.

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## References

[1] Anagnostaras SG, Dale GD, Fanselow MS. Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 2001;11:8–17.
[2] Anagnostaras SG, Josselyn SA, Frankland PW, Silva AJ. Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. Learn Mem 2000;7:58–72.
[3] Anagnostaras SG, Maren S, Fanselow MS. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci 1999;19:1106–14.
[4] Blanchard DC, Blanchard RJ. Cocaine potentiates defensive behaviors related to fear and anxiety. Neurosci Behav Rev 1999;23:981–91.
[5] Bolla KI, Cadet J, London ED. The neuropsychiatry of chronic cocaine abuse. J Neuropsychiat 1998;10:280–9.
[6] Bolla KI, Eldreth DA, London ED, Kiehl KA, Mouratidis M, Contoreggi C, et al. Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. Neuroimage 2003;19:1085–94.
[7] Bolla K, Ernst M, Kiehl K, Mouratidis M, Eldreth D, Contoreggi C, et al. Prefrontal cortical dysfunction in abstinent cocaine abusers. J Neuropsychiat Clin Neurosci 2004;16:456–64.
[8] Bolouri MR, Small GA. Neuroimaging of hypoxia and cocaine-induced hippocampal stroke. J Neuroimaging 2004;14:290–1.
[9] Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, et al. Neural systems and cue-induced cocaine craving. Neuropsychopharmacology 2002;26:376–86.
[10] Borowski TB, Kokkinidis L. The effects of cocaine, amphetamine, and the dopamine $D_1$ receptor agonist SKF 38393 on fear extinction as measured with potentiated startle: implications for psychomotor stimulant psychosis. Behav Neurosci 1998;112:952–65.
[11] Borowski TB, Kokkinidis L. Contribution of ventral tegmental area dopamine neurons to expression of conditional fear: effects of electrical stimulation, excitotoxin lesions, and quinpirole infusion on potentiated startle in rats. Behav Neurosci 1996;110:1349–64.
[12] Borowski TB, Kokkinidis L. Cocaine preexposure sensitizes conditioned fear in a potentiated acoustic startle paradigm. Pharmacol Biochem Behav 1994;49:935–42.
[13] Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, et al. Acute effects of cocaine on human brain activity and emotion. Neuron 1997;19:591–611.
[14] Breiter HC, Rosen BR. Functional magnetic resonance imaging of brain reward circuitry in the human. Ann NY Acad Sci 1999;877:523–47.
[15] Brice CF, Smith AP. Effects of caffeine on mood and performance: a study of realistic consumption. Psychopharmacology 2002;164:188–92.
[16] Cestari V, Mele A, Oliverio A, Castellano C. Amygdala lesions block the effect of cocaine on memory in mice. Brain Res 1996;713:286–9.
[17] Corodimas KP, Pruitt JC, Stieg JM. Acute exposure to caffeine selectively disrupts context conditioning in rats. Psychopharmacology 2000;152:376–82.
[18] Fanselow MS. Conditional and unconditional components of post-shock freezing. Pav J Biol Sci 1980;15:177–82.
[19] Fanselow MS, Poulos AM. The neuroscience of mammalian associative learning. Annu Rev Psychol 2005;56:207–34.
[20] Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. Neurosci Biobehav Rev 1999;23:743–60.
[21] Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, et al. The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 2005;30:296–309.
[22] Gale GD, Anagnostaras SG, Godsil BP, Mitchell S, Nozawa T, Sage JR, et al. Role of the basolateral amygdala in the storage of fear memories across the adult lifetime in rats. J Neurosci 2004;24:3810–5.
[23] Goldstein RZ, Leskovjan AC, Hoff AL, Hitzemann R, Bashan F, Khalsa SS, et al. Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. Neuropsychologia 2004;42:1447–58.
[24] Goto Y, Grace AA. Dopamine-dependent interactions between limbic and prefrontal cortical plasticity in the nucleus accumbens: disruption by cocaine sensitization. Neuron 2005;47:255–66.
[25] Grant S, Contoreggi C, London ED. Drug abusers show impaired performance in a laboratory test of decision making. Neuropsychologia 2000;38:1180–7.
[26] Haskell CF, Kennedy DO, Wesnes KA, Scholey AB. Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacology 2005;179:813–25.
[27] Hoff AL, Riordan H, Morris L, Cestaro V, Wieneke M, Alpert R, et al. Effects of crack cocaine on neurocognitive function. Psychiat Res 1996;60:167–76.
[28] Hyman SE. Addiction: a disease of learning and memory. Am J Psychiat 2005;162:1414–22.
[29] Jentsch JD, Olausson P, De La Garza R, Taylor JR. Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. Neuropsychopharmacology 2002;26:183–90.

[30] Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology 1999;146:373–90.
[31] Jovanovski D, Erb S, Zakzanis KK. Neurocognitive deficits in cocaine users: a quantitative review of the evidence. J Clin Exp Neuropsychol 2005;27:189–204.
[32] Kantak KM, Udo T, Ugalde F, Luzzo C, Di Pietro N, Eichenbaum HB. Influence of cocaine self-administration on learning related to prefrontal cortex or hippocampus functioning in rats. Psychopharmacology 2005;181:227–36.
[33] Liu Q, Pu L, Poo M. Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. Nature 2005;437:1027–31.
[34] Lundqvist T. Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to attention, memory and executive functions. Pharmacol Biochem Behav 2005;81:319–30.
[35] MacDonald Z, Pudney S. Illicit drug use, unemployment, and occupational attainment. J Health Econ 2000;19:1089–115.
[36] Mackowiak M, Markowicz-Kula K, Fijal K, Wedzony K. Acute and repeated administration of cocaine differentially regulated expression of PSA-NCAM-positive neurons in the rat hippocampus. Brain Res 2005;1055:149–55.
[37] Makris N, Gasic GP, Seidman LJ, Goldstein JM, Gastfriend DR, Elman I, et al. Decreased absolute amygdala volume in cocaine addicts. Neuron 2004;44:729–40.
[38] Maren S, Anagnostaras SG, Fanselow MS. The startled seahorse: is the hippocampus necessary for contextual fear conditioning? Trends Cogn Sci 1998;2:39–42.
[39] Overton DA. State-dependent learning produced by addicting drugs. In: Fisher C, Freedman AM, editors. Opiate addiction: origins and treatment. New York: Halsted Press Division of Wiley; 1972. p. 61–75.
[40] Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–85.
[41] Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 2004;47:33–46.
[42] Rogerio R, Takahashi RN. Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze. Pharmacol Biochem Behav 1992;43:631–3.
[43] Simon SL, Domier CP, Sim T, Richardson K, Rawson RA, Ling W. Cognitive performance of current methamphetamine and cocaine abusers. J Addict Dis 2002;21:61–74.
[44] Thompson AM, Gosnell BA, Wagner JJ. Enhancement of long-term potentiation in the rat hippocampus following cocaine exposure. Neuropharmacology 2002;42:1039–42.
[45] Thompson AM, Swant J, Gosnell BA, Wagner JJ. Modulation of long-term potentiation in the rat hippocampus following cocaine self-administration. Neuroscience 2004;127:177–85.
[46] Thompson AM, Swant J, Wagner JJ. Cocaine-induced modulation of long-term potentiation in the CA1 region of rat hippocampus. Neuropharmacology 2005;49:185–94.
[47] Trantham-Davidson H, Lavin A. Acute cocaine administration depresses cortical activity. Neuropsychopharmacology 2004;29:2046–51.
[48] Udo T, Ugalde F, DiPietro N, Eichenbaum HB, Kantak KM. Effects of persistent cocaine self-administration on amygdala-dependent and dorsal striatum-dependent learning in rats. Psychopharmacology 2004;174:237–45.
[49] van Duinen H, Lorist MM, Zijdewind I. The effect of caffeine on cognitive task performance and motor fatigue. Psychopharmacology 2005;180:539–47.
[50] van Gorp WG, Wilkins JN, Hinkin CH, Moore LH, Hull J, Homer MD, et al. Declarative and procedural memory functioning in abstinent cocaine abusers. Arch Gen Psychiat 1999;56:85–9.
[51] Warburton DM. Addiction controversies. Philadelphia: Harwood Academic Publishers; 1992. p. 23.
[52] Willick ML, Kokkinidis L. Cocaine enhances the expression of fear-potentiated startle: evaluation of state-dependent extinction and the shock-sensitization of acoustic startle. Behav Neurosci 1995;109:929–38.
[53] Yamaguchi M, Suzuki T, Seki T, Namba T, Juan R, Arai H, et al. Repetitive cocaine administration decreases neurogenesis in adult rat hippocampus. Ann NY Acad Sci 2004;1025:351–62.
[54] Yamaguchi M, Suzuki T, Seki T, Namba T, Liu J, Arai H, et al. Decreased cell proliferation in the dentate gyrus of rats after repeated administration of cocaine. Synapse 2005;58:63–71.
[55] Yang X, Gorman AL, Dunn AJ, Goeders NE. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. Pharmacol Biochem Behav 1992;41:643–50.

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Chapter 2

Psychopharmacology
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ORIGINAL INVESTIGATION

# Memory and psychostimulants: modulation of Pavlovian fear conditioning by amphetamine in C57BL/6 mice

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**Abstract**
*Rationale and objectives* With the use of prescription stimulants on the rise, it is important to examine the cognitive effects of low and moderate doses of stimulants rather than only those typical of addicts.
*Materials and methods* The present study examined the effects a range of doses (0.005–8 mg/kg) of D-amphetamine sulfate on cued and contextual Pavlovian fear conditioning in mice.
*Results* In agreement with previous research, subjects administered with a moderately high dose of amphetamine (8 mg/kg) pre-training, typical of what addicts might take, displayed impaired conditioned freezing when tested off-drug. Alternately, subjects injected with a very low dose of amphetamine (0.005, 0.025, or 0.05 mg/kg) pre-training, similar to the therapeutic doses for attention deficit hyperactivity disorder, displayed enhanced memory when tested off-drug. A control study showed that these effects were not due to state-dependent learning.
*Conclusions* Thus, dose is a critical determinant of the cognitive effects of psychostimulants.

**Keywords** Amphetamine · Fear conditioning · Learning and memory · Mouse

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## Introduction

Amphetamine can be highly addictive, leading individuals to exhibit behaviors ranging from relatively minor cognitive impairments to severe psychotic symptoms. Methamphetamine abusers display worse performance on tests of word recall, perceptual speed, vocabulary, and abstract reasoning, compared with controls (Simon et al. 2002). After prolonged amphetamine abuse, addicts may also exhibit symptoms similar to schizophrenia, commonly referred to as amphetamine or stimulant psychosis (Harris and Batki 2000).

Conversely, amphetamine is also used to treat ailments such as narcolepsy and attention deficit hyperactivity disorder (ADHD). Amphetamine (e.g., Adderall®) improves ADHD symptoms for most affected children (Ahmann et al. 2001), and several related stimulants are similarly effective (e.g., methamphetamine, methylphenidate, atomoxetine, modafinil; Leonard et al. 2004).

While prescription stimulants containing amphetamine have proven beneficial to many people suffering from ADHD, excessive daytime sleepiness, and narcolepsy, off-label use of these drugs is a growing problem. The life-time prevalence of non-medical use of prescription stimulants in college students has been recently reported to be 6.9% (McCabe et al. 2005) to 8.3% (Teter et al. 2006). These drugs tend to be regarded by the public as cognitive enhancers, presumably by promoting mental arousal or wakefulness. "Academic doping" is an emerging phenomenon in many educational settings (Butcher 2003). Stimulants also have documented benefits (and problems) in military use (Caldwell et al. 1995; Cornum et al. 1995; Cornum 1994). Indeed, a growing body of evidence demonstrates that healthy volunteers, not just those with ADHD, display cognitive benefits from low doses of stimulants (Barch and Carter 2005; Turner et al. 2003).

These trends illuminate the importance of studying the effects of a variety of acute doses of amphetamine rather than focusing solely on the higher, chronic doses typical of addicts. The current study examines the effects of a range of acute doses of D-amphetamine on a standard rodent learning and memory task, Pavlovian fear conditioning.

In Pavlovian fear conditioning, a discrete neutral stimulus (e.g., a tone) is paired with an aversive stimulus (e.g., a footshock). After training, when presented with the discrete stimulus, alone, the subject exhibits fear. In addition, upon returning to the environmental chamber in which it had been trained, the subject also exhibits fear, a phenomenon known as context conditioning. A common measure of conditioned fear in rodents is freezing, or the absence of all movements, excluding respiration (Fanselow 1980).

The neurobiology of Pavlovian fear conditioning has been studied extensively. The dorsal hippocampus is critically involved in encoding memory of the context in a time-graded manner (Anagnostaras et al. 1999, 2001). Because of the efficiency of contextual fear conditioning, it has become a leading model of explicit memory in rats and mice (Anagnostaras et al. 2000, 2001). The amygdala, and specifically the basolateral/lateral complex of the amygdala, is involved in encoding an aversive association with both the context and tone (Fanselow and Gale 2003; Fanselow and Poulos 2005). Amphetamine has been shown to alter amygdalar activity in a number of ways, including potentiating the synaptic transmission between the amygdala and nucleus accumbens (Kessal et al. 2005). The hippocampus has also been implicated in amphetamine-induced locomotor activity as lesions of the ventral hippocampus disrupt amphetamine-induced locomotion, and stimulation enhances it (White et al. 2006).

We previously found (Wood et al. 2007) that an acute dose of cocaine enhances learning on Pavlovian fear conditioning when given at a very low dose (0.1 mg/kg). Interestingly, we also found that a moderate dose of cocaine (15 mg/kg) disrupted conditioned freezing. These effects were general to both contextual and cued fear. These data reinforce the human findings that dosage is the critical determinant of whether a particular stimulant enhances or impairs memory. In particular, doses similar to those given for ADHD often improve attention, academic performance, and reduce impulsivity in humans. In contrast, high doses such as those taken by addicts are associated with unemployment, reduced executive function, anxiety, psychosis, and of course, addiction (Ellinwood et al. 1998). One potential confound of our prior study is that cocaine produces local anesthesia, which could directly reduce fear conditioning by reducing the painfulness of the footshock. Therefore, in the present study, we examined the effects of amphetamine on conditioned fear in order to extend the findings to a widely prescribed stimulant, as well as to control for local anesthesia. We also conducted a control study to address the issue of state-dependent effects on learning. We predicted that amphetamine would show the same pattern as cocaine, enhancing Pavlovian fear conditioning at low doses and interfering with fear conditioning at higher doses. The results of the current study are consistent with this hypothesis, showing enhanced learning during acquisition and recall of cued fear at low doses of amphetamine (0.005, 0.025, and 0.05 mg/kg) and deficits in conditioned freezing at higher doses (4 and 8 mg/kg). In addition, there was no evidence found to support the idea that state-dependent learning was a factor in the present results.

## Materials and methods

### Subjects

One-hundred thirty-six (68 male, 68 female) C57BL6/NCrl (B6; Charles River Laboratories, San Diego, CA, USA) mice were used for experiment 1, and 40 (19 male, 21 female) B6129SF1/J (H; Jackson Laboratory, West Sacramento, CA, USA) hybrid mice were used for experiment 2. Mice were weaned at 3 weeks of age and were group-housed (two to five mice per same sex cage), with continuous access to food and water. The animal colony was maintained on a 14:10 light/dark schedule, and all tests were performed during the light phase of the cycle. Mice were at least 8 weeks old before testing. All animal care and testing procedures were approved by the UCSD IACUC and were in accordance with the NIH "Principles of laboratory animal care." B6 and H mice were chosen because they display robust conditioned freezing and typical psychomotor reactivity to amphetamine (Anagnostaras et al. 2000; Yates et al. 2007). Finally, B6 and H mice are the most common background strains in studies using targeted mutations, and the present studies are part of a larger plan of study that involves several mutants (Crawley et al. 1997; Silva et al. 1997). H mice were used in experiment 2 due to availability but perform comparably on this task (Anagnostaras et al. 2003).

### Apparatus

*Conditioning context* Testing was performed in a windowless room. Background noise (65 dB) was provided by a HEPA air cleaner, and white light was provided by two 100-W bulbs. The mice were continuously recorded by a wall-mounted color video camera that was connected to a computer and video equipment in an adjacent room. Three to four mice were tested concurrently in individual conditioning chambers. Each chamber (32 cm wide, 25 cm high, 25 cm deep) was equipped with a stainless

steel grid floor (36 rods, each rod 2 mm in diameter, 8 mm center to center) and a speaker in the side wall. The side walls were white acrylic, and the front wall was clear to allow for viewing (Med-Associates Inc., St. Albans, VT, USA). A stainless steel drop-pan, scented with 7% isopropyl alcohol to provide a background odor, was located beneath each chamber. Between tests, the conditioning contexts were cleaned with 7% isopropyl alcohol solution. Each chamber was connected to a solid-state scrambler, providing AC constant current shock, and an audio stimulus generator was located in an adjacent room, controlled via an interface connected to a Windows computer running Med-PC (Med-Associates Inc., St Albans, VT, USA). Automated assessment of freezing and activity was provided by custom-designed software adapted from NIH Image running on an Apple Macintosh G4 (automated algorithm validated elsewhere; Anagnostaras et al. 2000).

*Alternate context* Multiple (three to four) mice were tested concurrently for tone fear in a separate room in individual boxes measuring 30 cm wide, 25 cm high, and 24 cm deep, and equipped with a speaker in the side walls. A clear Plexiglas front wall allowed the mice to be continually observed, while the ceiling, floor, and three interior walls of the chamber were solid white. To create a space distinct from the training context, a white plastic, triangular tent was placed inside each box; each side of the triangle measured 23 cm. Between tests, the chambers were cleaned and scented with a 5% white vinegar solution. The room was lit with dim red light, and an infrared video camera, connected to the Macintosh G4 described above, was used to score freezing.

*Drugs* Drugs were administered intraperitoneally (i.p.) in a volume of 5 or 10 ml/kg. D-Amphetamine sulfate (Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in 0.9% physiological saline. Amphetamine injections (salt weight; 0.005, 0.025, 0.05, 0.5, 1, 2, 4, or 8 mg/kg) were given 15 min before introduction to the testing equipment.

*Experiment 1: behavior measurement* In order to measure the effects of amphetamine on exploratory locomotor activity, baseline activity in 2 min before the first tone–shock pairing on the training day was assessed by counting the number of cross-overs each subject performed (Maren et al. 1998). A single cross-over was defined as the movement of a subject's entire body from one half of the box to the other. Videotapes of the conditioning sessions were observed using a standard VCR and monitor. In addition to number of cross-overs, exploratory activity was also measured using an automated, video-based activity measure (Anagnostaras et al. 2000).

To assess whether amphetamine disrupted shock reactivity, mouse activity burst displayed during the 2 s of shock exposure (unconditioned response to shock), as well as activity during the 2 s leading up to the shock, was measured as velocity (centimeter per second; Anagnostaras et al. 2000).

Experimental procedures

*Experiment 1*

*Conditioning* Mice were injected with saline or amphetamine 15 min prior to training. Training consisted of a 2-min baseline period, followed by three tone–shock pairings, each separated by 30 s. A tone–shock pairing consisted of a 30-s tone (2.8 kHz, 85 dB, A Scale), with a scrambled, constant current AC footshock (0.75 mA) administered during the last 2 s of the tone. Immediate post-shock freezing was measured for another 5 min. Thus, mice were inside the fear-conditioning chambers for a total of 10 min before being returned to their home cages.

*Testing* Mice were returned to the conditioning context without drug 24 h after training, and freezing was scored for 5 min. Mice were placed in the alternate context 48 h after training, also off-drug. Tone testing consisted of a 2-min baseline, followed by a continuous 3-min tone identical to the training tone. Freezing was scored for the entire 5-min period.

*Experiment 2*

Procedures for experiment 2 were identical to experiment 1, with the exception that amphetamine or saline injections were administered 15 min before testing, in addition to those administered before training.

## Results

Experiment 1

*Generalized activity* Amphetamine produced a dose-dependent increase in activity during the 2-min baseline period of training, measured both by an automated computer scoring system and by hand-counted cage cross-overs (Fig. 1a). Group differences in cage cross-overs were found on an analysis of variance [ANOVA; $F(8, 127)=16.5$, $p< 0.0001$]. Subjects administered with 4–8 mg/kg amphetamine pre-training displayed significantly more cross-overs during baseline than the saline control group [Fisher's protected least significant difference (PLSD) multiple post

hoc comparisons, $p$ values <0.01]. No other doses differed significantly from saline controls ($p$ values >0.05). An ANOVA demonstrated differences in the automated activity measure as well [$F(8, 127)=7.5$, $p<0.0001$]. As with the cross-over measures, only mice given 4–8 mg/kg of amphetamine showed a significant difference in activity from saline controls (Fisher's PLSD, $p$ values <0.01).

*Activity burst velocity* A large difference in velocity was elicited by the first 2-s shock presentation [unconditioned response (UR)], compared with the preceding 2 s, during which no shock was present (Fig. 1b). A multivariate ANOVA revealed a significant effect of dose on velocity [$F(8, 127)=2.3$, $p<0.05$], a significant effect of the shock [UR versus baseline; $F(1, 127)=1687.5$, $p<0.0001$], along with a significant time period by dose interaction [$F(8, 127)=2.5$, $p<0.05$], so the baseline and UR were considered separately. There were significant differences in the baseline velocity [$F(8, 127)=5.8$, $p<0.0001$]. Post hoc comparisons revealed similar differences in velocity as those seen in measurements of baseline activity, with subjects administered with 1, 4, or 8 mg/kg amphetamine displaying increased velocity compared with subjects administered with saline (Fisher's PLSD, $p$ values <0.05). No other doses differed significantly in terms of baseline activity from saline. There were also significant differences during the UR [$F(8, 127)=2.2$, $p<0.05$]. Only subjects given 0.025 or 8 mg/kg exhibited a larger UR to the shock compared to controls ($p$ values <0.05), but these differences were small and unrelated to the memory effects. Thus, amphetamine did not dull shock reactivity and seemed to enhance reactivity in certain cases.

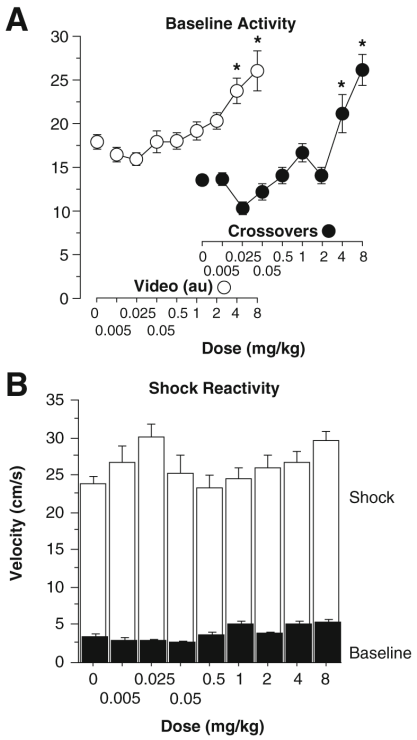


**Fig. 1** **a** Baseline activity. A dose-dependent increase in activity was observed during the 2-min baseline period prior to the first tone–shock pairing, with subjects administered with 4 or 8 mg/kg amphetamine prior to training showing greater activity than saline controls (*left*, an automated video activity measure and *right*, full cage cross-overs; mean ± SEM). *Asterisks* indicate a significant difference from saline controls. **b** Shock reactivity. All groups showed a significant increase in velocity (centimeter per second, mean ± SEM) during the 2-s shock compared with the 2-s baseline period leading up to the shock

*Training* Subjects were inside the conditioning contexts on the training day for a total of 10 min. Figure 2a depicts the first 5 min of training, consisting of a 2-min baseline period, followed by three tone–shock pairings during 3 min. A main effect for dose was present within the 5-min period [$F(8, 127)=36.5$, $p<0.0001$], as was a main effect for minute [$F(4, 127)=248.1$, $p<0.0001$], along with a significant dose by minute interaction [$F(32, 127)=15.4$, $p<0.0001$]. Post hoc comparisons revealed a dose-dependent effect of amphetamine on freezing, with subjects that had received 1, 2, 4, or 8 mg/kg freezing less than saline controls (Fisher's PLSDs, $p$ values <0.0001). Interestingly, subjects that had been administered with 0.005, 0.025, or 0.05 mg/kg amphetamine froze more than the saline control subjects ($p$ values <0.01). Subjects that received 0.5 mg/kg were not significantly different from saline controls ($p>0.05$).

*Post-shock freezing* In minutes 6 through 10 of training, freezing was measured with no further presentation of tone or shock, as an index of post-shock freezing (Fig. 2b). Dose-dependent differences in freezing were apparent [$F(8, 127)=68.7$, $p<0.0001$]. Post hoc tests revealed that subjects that had received 1, 2, 4, or 8 mg/kg amphetamine displayed less freezing than saline controls (Fisher's PLSD, $p$ values <0.001). As these subjects were still on-drug, it is important to note that amphetamine's locomotor effects could be influencing these results. Interestingly, however, subjects administered with 0.005, 0.025, or 0.05 pre-training again froze more than saline controls ($p$ values <0.05). Subjects that received 0.5 mg/kg were not significantly different from saline controls ($p>0.05$).

*Context fear* Subjects were returned to the conditioning chambers 24 h after training. Freezing was measured for 5 min, with all subjects off-drug (Fig. 3a). Dose-dependent differences in freezing were apparent [$F(8, 127)=20.7$, $p<0.0001$]. Subjects that had previously received 4 or 8 mg/kg

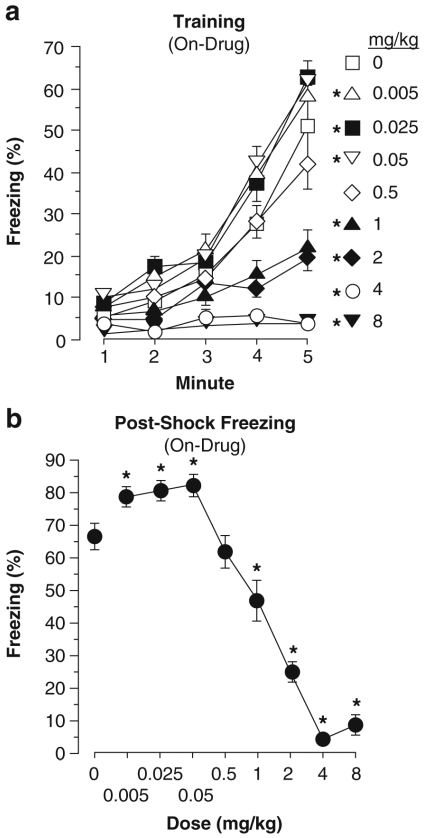


**Fig. 2** **a** Training. Freezing (mean ± SEM) of subjects on 1, 2, 4, or 8 mg/kg amphetamine was less than saline control subjects, while freezing of subjects on 0.005, 0.025, or 0.05 mg/kg amphetamine was greater than controls. **b** Post-shock freezing. Dose-dependent, post-training freezing was observed, with subjects on 0.005, 0.025, or 0.05 mg/kg amphetamine freezing more than controls, while those on 1, 2, 4, or 8 mg/kg amphetamine displayed less freezing than controls

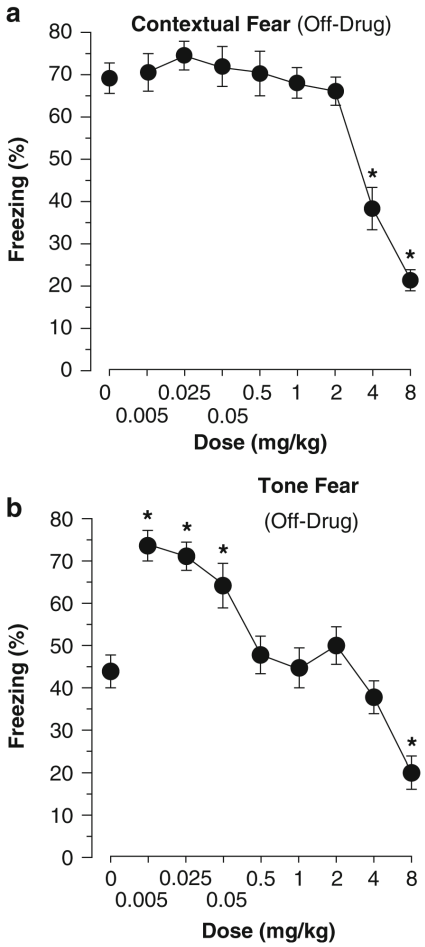


**Fig. 3** **a** Contextual fear. When returned to the training context 24 h after training off-drug, subjects that had received 4 or 8 mg/kg pre-training amphetamine displayed less freezing (mean ± SEM) than all other groups. **b** Tone fear. Freezing (mean ± SEM) during the 3-min period during the test when the tone was presented is depicted. Those that had been injected with 8 mg/kg pre-training displayed less freezing than all other groups, while those that had been administered with 0.005, 0.025, or 0.05 mg/kg displayed more freezing than all other groups

amphetamine before training displayed less freezing off-drug when reintroduced to the training context compared with saline controls (Fisher's PLSD, $p$ values <0.01). No other groups differed from saline controls ($p$ values >0.05).

*Tone fear* Subjects were introduced to a new context 48 h after training off-drug. Freezing was measured for 5 min, consisting of a 2-min baseline period and a 3-min tone presentation (Fig. 3b). The tone was the same frequency and volume as that which had been paired with the shock during training. Dose-dependent differences in freezing during the tone presentation were apparent [$F(8, 127)= 17.1$, $p<0.0001$]. Only subjects that had received 8 mg/kg amphetamine pre-training showed decreased freezing during the tone test compared with saline controls (Fisher's PLSD, $p<0.0001$). Those that had been injected with 0.005, 0.025, or 0.05 mg/kg amphetamine pre-training again showed increased freezing compared with saline controls ($p$ values <0.001). No other doses differed from saline controls ($p$ values >0.05).

*Experiment 1: summary* Overall, contextual fear memory tested off-drug was sensitive to disruption by 4–8 mg/kg of amphetamine, whereas tone fear was sensitive only to 8 mg/kg. An enhancement in cued, but not contextual, memory was observed at 0.005, 0.025, and 0.05 mg/kg.

Experiment 2

*Training and post-shock freezing* The training protocol for the state-dependent control study was the same as the dose–response study. Fifteen minutes after drug or saline injection, subjects were exposed to the conditioning chambers for a total of 10 min, composed of a 2-min baseline period, 3 min during which three tone–shock pairings were presented, and a 5-min immediate post-shock freezing test (Fig. 4a). Drug-dependent effects on freezing were evident [$F(3, 36)=88.7$, $p<0.0001$], with mice injected with saline pre-training showing markedly more freezing throughout training than those injected with 8 mg/kg amphetamine (Fisher's PLSD, $p$ values <0.0001). A main effect for training period was evident as well [$F(2, 36)=259.9$, $p<0.0001$], with average freezing increasing throughout training ($p$ values <0.0001). A significant drug by training period interaction [$F(6, 36)=59.7$, $p<0.0001$], however, demonstrated that this effect was driven differentially by the two saline groups, as the two groups on amphetamine did not show an increase in freezing.

*Context fear* Using the same protocol as experiment 1, subjects were returned to the conditioning chambers 24 h after training to measure contextual fear memory (Fig. 4b). Drug treatment had significant effects on contextual fear expression [$F(3, 36)=66.5$, $p<0.0001$], with both groups injected with 8 mg/kg amphetamine pre-testing displaying less freezing than those injected with saline (Fisher's PLSD, $p$ values <0.0001). Mice that received amphetamine during testing exhibited very little freezing and did not differ from one another ($p>0.05$). Consistent with experiment 1, mice that had been injected with 8 mg/kg amphetamine pre-training but then injected with saline pre-testing displayed less contextual freezing than those that had been injected with saline both pre-training and pre-testing ($p<0.0001$). Overall, no state-dependent learning effects were evident. Consistent with experiment 1, amphetamine was able to disrupt performance of freezing when animals were trained on-drug, as well as contextual freezing when those animals were tested 24 h later off-drug.

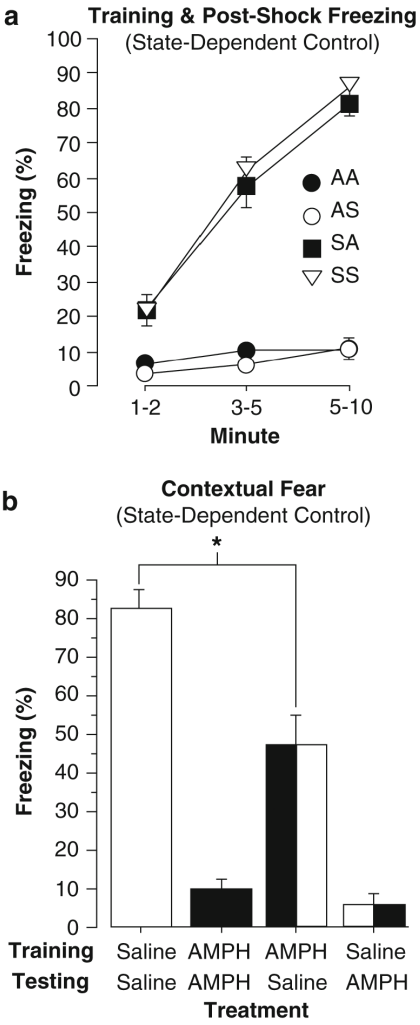


**Fig. 4** **a** State-dependent control—training and post-shock freezing. While subjects administered with saline before training showed a steady increase in freezing during training, subjects administered with 8 mg/kg amphetamine showed no increase in freezing. **b** State-dependent control—contextual fear. Regardless of treatment before training, subjects administered with 8 mg/kg amphetamine before contextual testing displayed very little freezing. In contrast, mice injected with saline before contextual testing showed some freezing if injected with 8 mg/kg before training and greater levels of freezing if injected with saline before training (*asterisk* indicates significant difference between these two groups). There was no evidence of state-dependent memory

## Discussion

We predicted that amphetamine would disrupt Pavlovian fear conditioning at moderate to high doses, similar to those that addicts take, and enhance fear conditioning at low doses, similar to those used to treat ADHD. As expected, moderate doses of amphetamine administered pre-training inhibited contextual and cue-elicited freezing off-drug, while very low doses enhanced cued fear memory. These data are in agreement with the results of our previous work with cocaine, which induced a similar pattern of enhancements and deficits in fear conditioning (Wood et al. 2007). A low dose of cocaine (0.1 mg/kg) produced an enhancement in contextual and cued fear, whereas a high dose

(15 mg/kg) produced an impairment in both measures. The combined results of these studies lead us to theorize that it is perhaps not the addictive properties of the drugs or the clinical profile of the subjects taking the drugs but, rather, the dose of drugs that lead to the divergent cognitive effects associated with psychostimulants.

One difference in the results of the present study and our previous research with cocaine (Wood et al. 2007) is that a range of low doses of amphetamine studied did not enhance contextual fear 24 h after training. It is unclear if the memory enhancement provided by a low dose of amphetamine is specific to cued fear as immediate post-shock freezing is believed to be a form of contextual fear (Fanselow 1986). Indeed, while low doses of amphetamine induced small but significantly higher levels of freezing during the immediate post-shock freezing test compared to saline controls, levels of freezing off-drug during the tone test were almost double that of controls. This evidence suggests the effects on locomotor activity and freezing are dissociable, especially for the low doses. It may be the case that some other, untested low dose of amphetamine would reveal enhanced memory on both contextual and cued tests off-drug, but it seems likely that a very low dose of amphetamine enhances cued memory, exclusively, since we tried a wide range of low doses (0.005–0.5 mg/kg) and induced large enhancements of tone memory. Another possibility is that the enhancement was not observed in contextual fear because of a "ceiling effect"; about 70% freezing is potentially the maximum that B6 mice may freeze under these training parameters (Anagnostaras et al. 2000, 2003). Clearly, a moderate or high dose of amphetamine disrupts conditioned freezing; there were some differences in terms of the deficits, in that 4 and 8 mg/kg produced significant deficits in contextual freezing, where only 8 mg/kg was effective in disrupting tone freezing. However, overall, the deficits in context and tone conditioning seem similar at high doses.

Research with a variety of human populations has produced mixed findings about the cognitive effects of stimulant use in humans. One study found cognitive benefits with amphetamine administration (0.25 mg/kg, orally) to people with schizophrenia already treated with antipsychotics (Barch and Carter 2005) despite the common notion that amphetamine exacerbates schizophrenia. The non-schizophrenic control subjects of the study, as well, exhibited improved language production and reaction time but had no benefit in working memory accuracy. In addition, it has been reported that low-dose amphetamine (10 mg, p.o., D-amphetamine, approximately 0.12 mg/kg for an 80-kg person) given during speech therapy leads to improved recovery from aphasia (Walker-Batson et al. 2001). These findings demonstrate the ability of stimulants to improve certain cognitive functions in a healthy population. Many studies have found cognitive deficits in amphetamine and cocaine addicts (Rogers and Robbins 2001). Currently using methamphetamine abusers were found to have worse performance on word recall, perceptual speed, an abbreviated IQ test, and the Wisconsin Card Sorting Task, compared with controls (Simon et al. 2002). In addition, methamphetamine addicts were found to perform more poorly on a decision-making task than their matched controls while displaying less activation in a number of frontal cortical areas, as measured by functional magnetic resonance imaging (fMRI; Paulus et al. 2002).

Previous research on amphetamine administration and associative learning in rodents has also yielded mixed findings. Rats administered with D-amphetamine (5 mg/kg, i.p.) or cocaine (40 mg/kg, i.p.) during extinction of fear-potentiated startle continued to show high startle amplitudes after 120 presentations of the nonreinforced CS (Borowski and Kokkinidis 1998). These results indicate that amphetamine and cocaine impaired the extinction of fear-potentiated startle. Extinction is generally conceived as the acquisition of new, inhibitory learning (Barad 2006). These doses of amphetamine and cocaine would produce impairments if given during training on our conditioned freezing task. Post-training injections of D-amphetamine at 1.0 mg/kg (i.p.), but not 0.25 or 4.0 mg/kg, have been shown to enhance inhibitory avoidance learning (Martinez et al. 1980). It is unclear if pre- versus post-training injections would share the same dose–response curve, so it is difficult to directly compare the results of the Martinez study with the current results. In addition, in order to examine amphetamine's effects on experience-dependent plasticity in rats, researchers examined fear conditioning in rats housed in a complex environment for 3 months (Briand et al. 2005). While rats showed enhanced learning after living in the complex environment, rats treated with D-amphetamine (4.0 mg/kg) for 21 days, then housed in the enriched environment for 3 months, did not show the same enhancement. The authors hypothesized that this moderate dose of amphetamine limited the experience-dependent structural plasticity normally engaged by a complex environment.

As with cocaine, several possible reasons exist as to why conditioned freezing was disrupted at high doses (Wood et al. 2007). While amphetamine does not have anesthetic properties, it could have induced a positive hedonic state in the mice. Those on higher doses may have perceived the shocks as less aversive than those on lower doses because of a drug-induced feeling of well being. While this experiment is not designed to fully explore this possibility, there is little evidence that this explanation accounts for the results. We measured the shock reactivity of subjects (Fig. 1b) and found that all subjects showed a large increase in velocity during the first shock presentation, in comparison to baseline velocity. Mice on 8 mg/kg

amphetamine seemed to perceive the shock at least as aversive as those on lower doses. In addition, this explanation of altered perception would imply that amphetamine produces a positive hedonic state disruptive to fear conditioning, whereas much research has shown that amphetamine is anxiogenic, enhancing most defensive behaviors in rodents (Markham et al. 2006).

Previous research on a range of doses of amphetamine supports an interpretation of attentional deficits disrupting performance. Rats showed impairment on a delayed matching-to-sample task at low doses of 0.6 and 1.0 mg/kg amphetamine, i.p. (Harper et al. 2005). At these doses, considered low doses for the present study on mice, the responses for a given trial were greatly influenced by responses from the preceding trial. These results were interpreted to stem from poor attention or confusion, as opposed to a pure associative memory deficit. Evidence for attentional deficits due to chronic amphetamine (5.0 mg/kg, i.p.) use was also found in extinction deficits of active and passive avoidance responses in mice (Kokkinidis 1983). Human studies, utilizing techniques such as fMRI (Paulus et al. 2002), have revealed frontal lobe dysfunction in amphetamine-dependent individuals, resulting in poor attention and decision making. Taken together, there is evidence from previous work that amphetamine could be hindering performance on fear conditioning due to attentional rather than learning deficits.

An additional, potential confound to be addressed is state-dependent learning. According to this theory, subjects on-drug during training would remember what they learned better if also tested on-drug. A discrepancy in drug state between training and testing has been shown to be detrimental to learning, for certain drugs. For example, one representative study examined the effects of benzodiazepine [chlordiazepoxide (CDP)] administration during extinction of fear conditioning (Bouton et al. 1990). They found that extinction was state dependent when done on CDP. However, in the present study using amphetamine, we found no evidence of state-dependent learning (Fig. 4). In the present study, subjects that received 0.005, 0.025, or 0.05 mg/kg amphetamine pre-training displayed higher levels of freezing than controls when tested off-drug. This finding contradicts the prediction of state-dependent learning. In addition, the immediate post-shock freezing test, performed on-drug, elicited a greater deficit in freezing than when subjects were tested off-drug. While an immediate memory test for a study of this design is clearly confounded with the motor stimulant properties of amphetamine, it is worthy to note that animals administered with a high dose of amphetamine during both testing and training showed no increase in freezing over those that had received drug only during testing in experiment 2. Indeed, subjects given amphetamine during both training and testing exhibited almost no evidence of learning, suggesting that there was no state-dependent learning. Rather, amphetamine appears to have disrupted performance of the freezing response when given during testing and not training and disrupted the acquisition of fear conditioning when given during training and not testing. Interpretation of this data, thus, is somewhat problematic since high-dose amphetamine may have had two separate actions: disrupting memory formation and/or disrupting freezing by producing hyperactivity. Overall, however, amphetamine and other stimulants have not been shown to produce a high level of state-dependent learning compared with other addictive drugs (Overton 1972).

Studies on the effects of amphetamine on long-term potentiation (LTP), an experience-dependent increase in synaptic efficacy thought to be associated with learning, have produced conflicting results. Broadly, some research on methamphetamine users has shown a decreased density of dopamine transporter in the striatum (Lundqvist 2005). As activation of DA receptors can modulate the expression of glutamate receptors, DA receptor activity can, in turn, alter synaptic plasticity (Sun et al. 2005). LTP in the dentate gyrus was also found to be enhanced by amphetamine in a dose-dependent manner (Gold et al. 1984). Increased potentiation of population spike amplitude was found in rats that had been administered with 0.01, 0.1, 1.0, or 3.0 mg/kg amphetamine, i.p., while doses higher (10.0 mg/kg) and lower (0.001 mg/kg) produced no differences compared to controls. In other research, however, acute amphetamine administration (5.0 mg/kg, i.p.) did not alter perforant path LTP of EPSP slope but caused a small reduction in LTP of the population spike amplitude (Morimoto et al. 1987). The authors interpreted these results as showing amphetamine reduces cellular excitability. LTP was blocked in nucleus accumbens neurons when slices were bathed in 2.5 μM amphetamine solution (Li and Kauer 2004). Interestingly, this effect was attenuated when rats were administered with amphetamine (2.5 mg/kg, i.p.) in vivo for 6 days, and nucleus accumbens slices were prepared 8–10 days after the last injection. Additionally, a higher AMPA-receptor/NMDA-receptor ratio at the glutamatergic synapses in the ventral tegmental area (VTA) was associated with higher behavioral sensitization to a single dose of amphetamine in young rats (Faleiro et al. 2004). These results indicate that LTP occurred rapidly in the VTA and with a single amphetamine exposure. Overall, studies of amphetamine and synaptic plasticity have yielded mixed results but demonstrate that low doses of amphetamine appear to enhance cell excitability and synaptic plasticity.

In general, research in humans and rodents on memory and synaptic plasticity is in agreement with the current study for acute use of amphetamine, suggesting that very

low doses are beneficial to cognition while moderate or high doses are detrimental. We have previously found similar results with cocaine (Wood et al. 2007). The implications of this research are broad, ranging from addicts' use to students using stimulants for academic doping and those prescribed stimulants for learning disabilities. An enhanced understanding of when these drugs produce improvements in cognition, as opposed to deficits and addiction, is needed. The present study suggests that dosing is a crucial determinant of stimulant effects on cognition. At very low doses, cognition is enhanced, whereas high doses promote addiction and disrupt cognition. This hypothesis seems more parsimonious, given the sum of the data, than other theories that posit that stimulants act differently in children versus adults or in subjects with or without ADHD (Marx 1999).

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## References

Ahmann PA, Theye FW, Berg R, Linquist AJ, Van Erem AJ, Campbell LR (2001) Placebo-controlled evaluation of amphetamine mixture-dextroamphetamine salts and amphetamine salts (Adderall): efficacy rate and side effects. Pediatrics 107:E10

Anagnostaras SG, Maren S, Fanselow MS (1999) Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci 19:1106–1114

Anagnostaras SG, Josselyn SA, Frankland PW, Silva AJ (2000) Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. Learn Mem 7:58–72

Anagnostaras SG, Gale GD, Fanselow MS (2001) Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 11:8–17

Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, Silva AJ (2003) Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci 6:51–58

Barad M (2006) Is extinction of fear erasure or inhibition? Why both, of course. Learn Mem 13:108–109

Barch DM, Carter CS (2005) Amphetamine improves cognitive function in medicated individuals with schizophrenia and in healthy volunteers. Schizophr Res 77:43–58

Borowski TB, Kokkinidis L (1998) The effects of cocaine, amphetamine, and the dopamine D1 receptor agonist SKF 38393 on fear extinction as measured with potentiated startle: implications for psychomotor stimulant psychosis. Behav Neurosci 112:952–965

Bouton ME, Kenney FA, Rosengard C (1990) State-dependent fear extinction with two benzodiazepine tranquilizers. Behav Neurosci 104:44–55

Briand LA, Robinson TE, Maren S (2005) Enhancement of auditory fear conditioning after housing in a complex environment is attenuated by prior treatment with amphetamine. Learn Mem 12:553–556

Butcher J (2003) Cognitive enhancement raises ethical concerns. Academics urge pre-emptive debate on neurotechnologies. Lancet 362:132–133

Caldwell JA, Caldwell JL, Crowley JS, Jones HD (1995) Sustaining helicopter pilot performance with Dexedrine during periods of sleep deprivation. Aviat Space Environ Med 66:930–937

Cornum K, Cornum R, Storm W (1995) Use of psychostimulants in extended flight operations: a Desert Shield experience Aerospace Medical Panel Symposium (Advisory Group for Aerospace Research and Development Conference Proceedings 579, Neurological Limitations of Aircraft Operations: Human Performance Implications). Koln, Germany, pp 371–374

Cornum KG (1994) Extended air combat operations: F-15s over Iraq. Aerospace Medical Association 65th Annual Scientific Meeting. Aerospace Medical Association, Washington, p A49

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ, Maxson SC, Miner LL, Silva AJ, Wehner JM, Wynshaw-Boris A, Paylor R (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 132:107–124

Ellinwood EH, King GR, Lee TH (1998) Chronic amphetamine use and abuse. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress. CD Rom Version. 4th edn. Lippincott, Williams and Wilkins, Philadelphia

Faleiro LJ, Jones S, Kauer JA (2004) Rapid synaptic plasticity of glutamatergic synapses on dopamine neurons in the ventral tegmental area in response to acute amphetamine injection. Neuropsychopharmacology 29:2115–2125

Fanselow MS (1980) Conditioned and unconditional components of post-shock freezing. Pavlov J Biol Sci 15:177–182

Fanselow MS (1986) Associative vs. topographical accounts of the immediate shock freezing deficit in rats: implications for the response selection rules governing species specific defensive reactions. Learning & Motivation 17:16–39

Fanselow MS, Gale GD (2003) The amygdala, fear, and memory. Ann NY Acad Sci 985:125–134

Fanselow MS, Poulos AM (2005) The neuroscience of mammalian associative learning. Annu Rev Psychol 56:207–234

Gold PE, Delanoy RL, Merrin J (1984) Modulation of long-term potentiation by peripherally administered amphetamine and epinephrine. Brain Res 305:103–107

Harper DN, Wisnewski R, Hunt M, Schenk S (2005) (+/−)3,4-methylenedioxymethamphetamine, D-amphetamine, and cocaine impair delayed matching-to-sample performance by an increase in susceptibility to proactive interference. Behav Neurosci 119:455–463

Harris D, Batki SL (2000) Stimulant psychosis: symptom profile and acute clinical course. Am J Addict 9:28–37

Kessal K, Chessel A, Spennato G, Garcia R (2005) Ketamine and amphetamine both enhance synaptic transmission in the amygdala-nucleus accumbens pathway but with different time-courses. Synapse 57:61–65

Kokkinidis L (1983) The effects of chronic amphetamine administration on the acquisition and extinction of an active and passive avoidance response in mice. Pharmacol Biochem Behav 19:593–598

Leonard BE, McCartan D, White J, King DJ (2004) Methylphenidate: a review of its neuropharmacological, neuropsychological and adverse clinical effects. Hum Psychopharmacol 19:151–180

Li Y, Kauer JA (2004) Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. Synapse 51:1–10

Lundqvist T (2005) Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to

attention, memory and executive functions. Pharmacol Biochem Behav 81:319–330

Maren S, Anagnostaras S, Fanselow M (1998) The startled seahorse: is the hippocampus necessary for contextual fear conditioning? Trends Cogn Sci 2:39–42

Markham CM, Yang M, Blanchard RJ, Blanchard DC (2006) Effects of D-amphetamine on defensive behaviors related to fear and anxiety. Pharmacol Biochem Behav 83:490–499

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 Res 182:157–166

Marx J (1999) How stimulant drugs may calm hyperactivity. Science 283:306

McCabe SE, Knight JR, Teter CJ, Wechsler H (2005) Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. Addiction 100:96–106

Morimoto K, Otani S, Goddard GV (1987) Effects of acute and long-term treatment with amphetamine on evoked responses and long-term potentiation in the dentate gyrus of anesthetized rats. Brain Res 407:137–143

Overton DA (1972) State-dependent learning produced by addicting drugs. In: Fisher C, Freedman AM (eds) Opiate addiction: origins and treatment. Halsted Press Division of Wiley, New York, pp 61–75

Paulus MP, Hozack NE, Zauscher BE, Frank L, Brown GG, Braff DL, Schuckit MA (2002) Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. Neuropsychopharmacology 26:53–63

Rogers RD, Robbins TW (2001) Investigating the neurocognitive deficits associated with chronic drug misuse. Curr Opin Neurobiol 11:250–257

Silva AJ, Simpson EM, Takahashi JS, Lipp H, Nakanishi S, Wehner JM, Giese KP, Tully T, Abel T, Chapman PF, Fox K, Grant S, Itohara S, Lathe R, Mayford M, McNamara JO, Morris RJ, Picciotto M, Roder M, Shin H, Slesinger PA, Storm DR, Stryker MP, Tonegawa S, Wang Y, Wolfer DP (1997) Mutant mice and neuroscience: recommendations concerning genetic background. Banbury Conference on genetic background in mice. Neuron 19:755–759

Simon SL, Domier CP, Sim T, Richardson K, Rawson RA, Ling W (2002) Cognitive performance of current methamphetamine and cocaine abusers. J Addict Dis 21:61–74

Sun X, Zhao Y, Wolf ME (2005) Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. J Neurosci 25:7342–7351

Teter CJ, McCabe SE, LaGrange K, Cranford JA, Boyd CJ (2006) Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. Pharmacotherapy 26:1501–1510

Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ (2003) Cognitive enhancing effects of modafinil in healthy volunteers. Psychopharmacology (Berl) 165:260–269

Walker-Batson D, Curtis S, Natarajan R, Ford J, Dronkers N, Salmeron E, Lai J, Unwin DH (2001) A double-blind, placebo-controlled study of the use of amphetamine in the treatment of aphasia. Stroke 32:2093–2098

White IM, Whitaker C, White W (2006) Amphetamine-induced hyperlocomotion in rats: hippocampal modulation of the nucleus accumbens. Hippocampus 16:596–603

Wood SC, Fay J, Sage JR, Anagnostaras SG (2007) Cocaine and Pavlovian fear conditioning: dose–effect analysis. Behav Brain Res 176:244–250

Yates JW, Meij JT, Sullivan JR, Richtand NM, Yu L (2007) Bimodal effect of amphetamine on motor behaviors in C57BL/6 mice. Neurosci Lett 427:66–70

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# Chapter 3

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## Amphetamine and extinction of cued fear

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ABSTRACT

Much research is focused on developing novel drugs to improve memory. In particular, psychostimulants have been shown to enhance memory and have a long history of safe use in humans. In prior work, we have shown that very low doses of amphetamine administered before training on a Pavlovian fear-conditioning task can dramatically facilitate the acquisition of cued fear. The current experiment sought to expand these findings to the extinction of cued fear, a well-known paradigm with therapeutic implications for learned phobias and post-traumatic stress disorder. If extinction reflects new learning, one might expect drugs that enhance the acquisition of cued fear to also enhance the extinction of cued fear. This experiment examined whether 0.005 or 0.05 mg/kg of D-amphetamine (therapeutic doses shown to enhance acquisition) also enhance the extinction of cued fear. Contrary to our hypothesis, amphetamine did not accelerate extinction. Thus, at doses that enhance acquisition of conditioned fear, amphetamine does not appear to enhance extinction.



A large body of evidence suggests that psychostimulants can enhance learning and memory in both humans and rodents [6,21,30,31,33]. One such psychostimulant is amphetamine, a drug currently used to treat attention deficit hyperactivity disorder (ADHD; e.g. Adderall®) [1]. Our laboratory has previously found [37] that ultra-low doses (0.005 and 0.05 mg/kg) of amphetamine, similar to the therapeutic doses for ADHD, administered to mice during training, dramatically enhance cued fear memory when subjects are tested off-drug. It is clear that amphetamine can enhance the acquisition of aversive memories, but it is unclear whether amphetamine can also enhance the extinction of conditioned fear.

In Pavlovian fear conditioning, an initially neutral stimulus (the conditioned stimulus, CS, e.g. a tone) is paired with an aversive stimulus (the unconditioned stimulus, US, e.g. a footshock). Following repeated CS–US pairings, the CS alone can elicit fear in a subject. In rodents, freezing, or the absence of all movement with the exception of respiration, is often the measure of conditioned fear [2,12]. The neurobiology underlying conditioned freezing is well understood; acquisition of cued fear depends critically on the convergence of CS and US information in the basolateral amygdala [20,28]. This CS–US association is not necessarily permanent, however. Repeated presentations of the CS in the absence of the US lead to extinction of conditioned fear, evidenced by decreased freezing in response to the CS alone.

Extinction is thought to reflect new, inhibitory learning [24], whereby extinction training encodes a new memory of the CS that then competes with the original memory of the CS. Unlike acquisition of cued fear, the neural mechanisms underlying extinction are still poorly understood. For example, extinction seems to depend on the medial prefrontal cortex (which is not essential for fear acquisition) [22,25], as well as the amygdala [5,11].

Pavlovian fear conditioning can serve as a model for both the etiology and treatment of phobia because phobias, or maladaptive fear responses to conditioned stimuli [36], are frequently treated using extinction therapy [13,14]. Extinction, however, is a relatively weak and unstable form of learning, so considerable research has focused on identifying pharmacological agents, which, if given during extinction therapy would strengthen and stabilize the reduction of fear [27,35]. Therefore, if extinction reflects new, inhibitory learning, it is possible that drugs that enhance fear acquisition will also facilitate the extinction of fear memory. This study examined whether extinction could be facilitated using D-amphetamine, a psychostimulant drug previously shown to enhance acquisition of cued fear [37].

The effects of amphetamine on the extinction of conditioned freezing have only been examined in one other study. Mueller et al. [23] administered 1.0 mg/kg of amphetamine during extinction training. They found that amphetamine decreased freezing relative to saline controls during extinction training, but this effect was not seen when tested off-drug. Thus, they attributed the reduction in freezing to amphetamine-induced locomotor hyperac-

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tivity rather than enhanced extinction retention. Mueller's results are not surprising in light of our recent findings, which found evidence for hyperactivity and no evidence of memory enhancement in animals administered 1 mg/kg D-amphetamine [37]. Only ultra-low doses of amphetamine (0.005–0.05 mg/kg) administered pre-training enhanced cued fear acquisition. Thus, these ultra-low doses of amphetamine are more likely than the moderate dose to enhance the extinction of Pavlovian fear-conditioning. Therefore, we administered 0.005 and 0.05 mg/kg amphetamine during extinction training and found that neither dose altered the extinction of Pavlovian fear.

Fifty-two C57B6/J inbred mice from Jackson Laboratory (West Sacramento, CA) were used in approximately equal numbers of males and females, balanced across groups. Mice were weaned at 3 weeks of age and were group housed (2–5 mice per cage) with continuous access to food and water. Mice were at least 10 weeks old before testing and subjects were handled for 5 days prior to training. The vivarium was maintained on a 14:10 light:dark schedule, and all testing was performed during the light phase of the cycle. All animal care and testing procedures were approved by the UCSD IACUC and were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

Mice underwent acquisition training (tone–shock pairings) for 1 day, off-drug, followed by 6 days of extinction trials (tone-alone presentations) under saline or amphetamine conditions. One final day of extinction was conducted off-drug. Three to four mice were tested concurrently, in individual conditioning chambers housed in a windowless room. Conditioning chambers were setup as described previously [29,37]. Each conditioning chamber (32 cm × 25 cm × 25 cm) was located within a sound-attenuating chamber (63.5 cm × 35.5 cm × 76 cm) (Med-Associates Inc., St. Albans, VT) and equipped with a speaker in the sidewall. During acquisition training, the context consisted of a stainless steel grid floor (36 rods, each rod 2 mm in diameter, 8 mm center to center; Med-Associates Inc., St. Albans, VT) and a stainless steel drop pan. The sidewalls were white acrylic, and the front wall was clear to allow for viewing. Between each trial, the chambers were cleaned and scented with 7% isopropyl alcohol to provide a background odor. Ventilation fans provided background noise (65 dB). Each sound-attenuating chamber was equipped with an overhead LED light source, providing white and near-infrared light. The mice were continuously observed by a wall-mounted IEEE 1394 progressive scan video camera with a visible light filter (VID-CAM-MONO-2A; Med-Associates Inc., St. Albans, VT) connected to a computer in an adjacent room. Each chamber was connected to a solid-state scrambler, providing AC constant current shock, and an audio stimulus generator, controlled via an interface connected to a Windows computer running Video Freeze (Med-Associates, Inc., St. Albans, VT), a program designed for the automated assessment of freezing and locomotor activity. In results that will be published more fully elsewhere, computer and human scored data had a correlation of 0.971 and a fit of computer = −.007 + .974 × human (for more detail on this calculation, see [3]).

The conditioning context was altered along several dimensions for the extinction trials. White acrylic sheets were placed over the grid floors and a black plastic, triangular tent (23 cm, each side), translucent to near-infrared light, was placed inside each box. Only near-infrared light was used, creating a dark environment visible only to the video camera. Between extinction trials, the chambers were cleaned and scented with a 5% vinegar solution.

Acquisition training was conducted off-drug and consisted of a 2-min baseline activity period, followed by 9 tone–shock pairings, each separated by 20 s. During each tone–shock pairing, a 10-s tone (conditioned stimulus: 2.8 kHz, 90 dB, A scale) was presented and co-terminated with a scrambled footshock (unconditioned stimulus: 2 s, 0.75 mA, AC constant current) delivered through the floor of the cages. Freezing behavior, defined as the absence of all movement with the exception of respiration [12], was scored automatically using Video Freeze software (Med-Associates, Inc., St. Albans, VT). Mice were inside the fear-conditioning chambers for a total of 9 min before being returned to their home cages.

Twenty-four hours after training, mice began the first of 6 days of extinction trials in the alternate context described above, on-drug. Extinction consisted of a 1-min baseline, followed by 15 presentations of the training tone (10-s tone, 20-s interval between tones). Mice were removed from the chambers 30 s later and returned to their home cages. Freezing and activity were scored for the entire 9-min period during each extinction day. Drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. D-Amphetamine hemisulfate (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in 0.9% sodium chloride. Amphetamine injections (salt weight: 0.005 or 0.05 mg/kg) were given i.p. 15 min prior to extinction trials. Mice were randomly assigned to one of three groups indicating the amount of amphetamine administered: 0 mg/kg (saline control, $n = 20$), 0.005 mg/kg ($n = 16$), and 0.05 mg/kg amphetamine ($n = 16$). Doses were chosen based on a previous study of cued fear acquisition [37]. A single, additional day of extinction (Day 7) was conducted off-drug, to serve as a state-dependent control.

Fig. 1 depicts each minute of acquisition training, consisting of a 2-min baseline period, followed by 9 tone–shock pairings, and a 2.5-min post-shock period. There was a main effect for minute [$F(8,392) = 66.1$, $p < 0.0001$], with freezing increasing after the onset of the tone–shock pairings. The animals were off-drug and no group differences [$F(2,49) = 0.819$, $p = 0.447$] or group by minute interactions [$F(2,49) = 0.388$, $p = 0.681$] were observed. On the first day of extinction, baseline locomotor activity (measured in arbitrary units by an automated computer scoring system) did not differ between groups [$F(2,49) = 0.156$, $p = 0.856$], suggesting that the low doses of amphetamine did not influence locomotor activity (data not depicted; see also [37]).

As we were interested in examining between-trial extinction (extinction retention [24]) and not within-trial extinction, we calculated the average freezing during the first 5 tones each day (Fig. 2A). Between-trial extinction seems more relevant to the treatment of learned fear because it is long lasting. We encountered moderately high baseline freezing during each extinction session (Fig. 2A, dashed lines), so we also measured tone freezing by subtracting baseline freezing from tone-elicited freezing (Fig. 2B). Subjects underwent 6 days of extinction trials (on-drug), and a

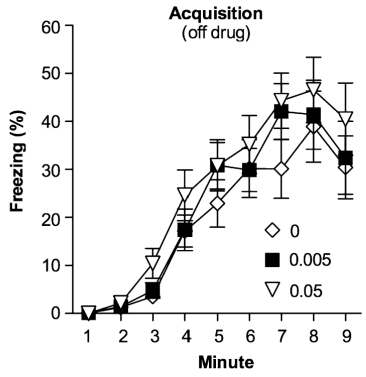


**Fig. 1.** Percentage of time spent freezing during training. The shocks were presented starting at 2 min. All subjects were off-drug and all groups showed the same freezing behavior. Each group represents the dose (mg/kg) of amphetamine given prior to each extinction trial (not given during acquisition). Each point represents the $M \pm SEM$.

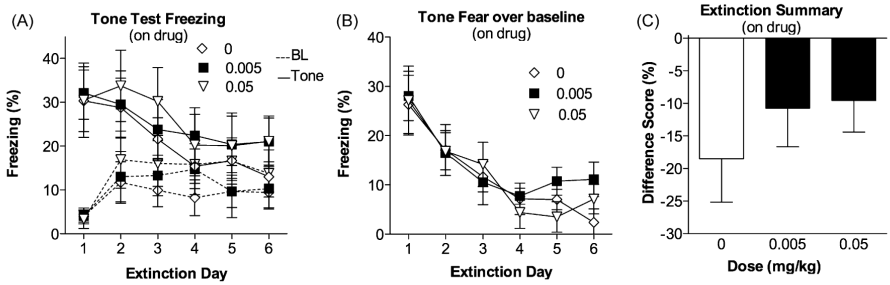


**Fig. 2.** (A) Percentage of time spent freezing during baseline (BL) and the average of the first 5 tone presentations (Tone) for each of the six on-drug extinction trial days. Each group represents the dose (mg/kg) of amphetamine given prior to each extinction trial. (B) Percentage of time spent freezing during the first tone block (first 5 tone presentations averaged) over baseline for each extinction day. (C) Difference between the percentages time spent freezing over baseline during the first tone block (first 5 tone presentations) on extinction Day 6 and extinction Day 1. All groups show evidence of extinction. Amphetamine did not affect between-trial or overall extinction. Each point represents the $M \pm SEM$.

main effect of day on average freezing over baseline during the first 5 tones was present [$F(5,245) = 15.890$, $p < 0.0001$] (Fig. 2B). Cued fear decreased as the number of extinction trials increased. Thus, all of the groups showed cued fear extinction; freezing decreased by at least 50% between Days 1 and 6 of the extinction trials. No group differences in between-trial extinction [$F(2,49) = 0.223$, $p = 0.801$] or group-by-day interaction [$F(10,245) = 0.498$, $p = 0.89$] were observed. To purely measure extinction, we generated a difference score by subtracting average freezing during the first 5 tones of extinction Day 1 from average freezing during the first 5 tones of extinction Day 6 (Fig. 2C). Again, all of the groups showed extinction, as demonstrated by the negative difference scores (percent freezing was greater on Day 1 than on Day 6 for all groups). No group differences were observed [$F(2,49) = 0.280$, $p = 0.757$]. Finally, although this experiment was not optimally designed to examine within-trial extinction because of the very close spacing of the tone presentations, no group differences were found in terms of short-term extinction during extinction Day 1 across the 15 tones [MANOVA, group by time interaction $F(2,49) = 0.81$, $p = 0.738$, or the difference between the average of tones 1–3 and 13–15, $F(2,49) = 0.925$, $p = 0.404$; data not depicted].

We also examined locomotor activity during the extinction trials as an alternate index of fear [3]. As in our previous analyses, we examined activity across the first 5 tone presentations to compare between-trial, rather than within-trial, changes in activity. We generated a suppression ratio to control for baseline differences in subjects' activity. The suppression ratio was defined as: (average activity during the first 5 tones)/(activity during the first 5 tones + activity during extinction trial baseline). Very low values indicate a high level of fear, 0.5 indicates no fear, and values greater than 0.5 can indicate conditioned safety [3,4]. There was a significant effect of day on the activity suppression ratios [$F(5,245) = 27.102$, $p < 0.0001$] (Fig. 3A), with suppression scores increasing (indicating decreased fear) as the number of extinction trials increased. By extinction Day 6, the suppression ratios were significantly larger (indicating less fear) than they had been on Day 1. No main effect of group [$F(2,49) = 0.337$, $p = 0.715$], or day-by-group interaction [$F(10,245) = 1.09$, $p = 0.370$] was observed.

On the last extinction day (Day 7), subjects underwent the same extinction protocol as Days 1–6, but were tested off-drug. This trial served as a state-dependent control. Regardless of treatment on prior extinction trial days, subjects displayed low levels of freezing when tested off-drug; tone-elicited freezing (average of the 5 tones) minus baseline freezing (first minute) is depicted (Fig. 3B). The extinction memory was retained and there was no evidence of state-dependent memory. No group differences in tone-elicited freezing were found [$F(2,49) = 0.007$, $p = 0.993$]. These results provide no evidence that amphetamine altered the extinction of cued fear.

We examined the effects of amphetamine on the extinction of cued fear. As has been reported with higher doses [23], we found that low (therapeutic) doses of amphetamine do not facilitate extinction of conditioned fear. We hypothesized that because cued fear extinction involves new learning, ultra-low doses of

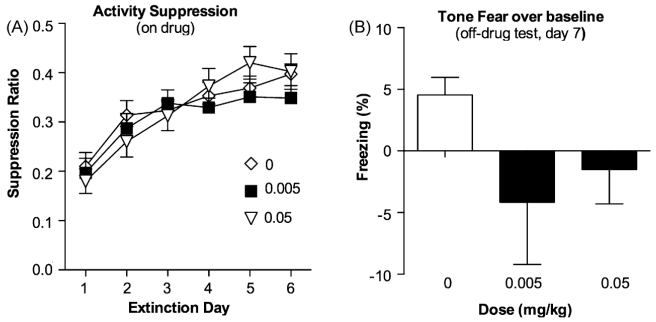


**Fig. 3.** (A) Activity suppression for each of the six on-drug extinction trial days. Activity suppression was computed as suppression ratio = (average activity during the first 5 tones)/(activity during the first 5 tones + activity during baseline). Values close to 0.0 reflect high levels of fear; values close to 0.5 reflect no fear [4]. Amphetamine administered before each extinction trial did not affect activity suppression. Each point represents the $M \pm SEM$. (B) Percentage time spent freezing during the state-dependent control test (extinction Day 7). All animals were off-drug and there was no evidence of state-dependent memory. Each bar represents the $M \pm SEM$.

amphetamine, previously shown to dramatically enhance cued fear acquisition [37], would also enhance extinction. Mueller et al. [23] failed to observe a facilitatory effect of amphetamine on cued fear extinction, perhaps because they used a dose (1 mg/kg) that does not affect cued fear acquisition [37]. Our results, however, are not consistent with this hypothesis.

Prior research has also found that amphetamine does not affect extinction on other behavioral paradigms. For example, a moderately high dose of amphetamine (5 mg/kg) given during extinction of fear-potentiated startle in rats failed to alter extinction [8]. Also, amphetamine (1 mg/kg) had no effect on extinction of conditioned approach [7,10]. Amphetamine (5 mg/kg) has even been found to impair extinction of passive avoidance [15,16]. As with Mueller et al. [23], however, all of these studies used moderate to high doses of amphetamine that induce locomotor hyperactivity and impair the acquisition of fear-conditioning [37]. Thus, to address this confound we used very low doses of amphetamine that do not influence activity, but can enhance memory [37]. As expected, baseline activity measurements during the first day of extinction did not differ between the amphetamine and saline groups. Thus, amphetamine's lack of effect on extinction in the current experiment cannot be attributed to amphetamine-induced alterations in locomotor activity.

One explanation for our finding is that the acquisition of aversive memories and their extinction reflect different types of new memory formation. Early evidence that N-methyl-D-aspartate (NMDA) receptors are essential for both acquisition and extinction of fear fostered enthusiasm that the mechanisms of acquisition and extinction may be similar [19,34,35]. However, more recent evidence suggests that the neural circuitry and pharmacology of fear acquisition and extinction are dissociable [for a review see [24,26]]. Li et al. [18] provide a model demonstrating how the amygdala could encode fear acquisition and extinction memories independently using discrete neural pathways. At the synaptic level, extinction, but not acquisition, depends on cannabinoid receptors [32]. At the systems level, extinction, but not acquisition, may depend on the medial prefrontal cortex [22,25]. If the neural mechanisms were different, then a drug would not necessarily be expected to enhance both acquisition and extinction. Additionally, acquisition and extinction may have different dose–response curves for pharmacological manipulation, though this seems unlikely as 1.0 mg/kg [23], and now 0.005 and 0.05 mg/kg, amphetamine has been shown to have no effect on cued fear extinction.

Several limitations in this study need to be addressed. The mice showed somewhat low levels of freezing to the tone on the first day of extinction (about 30%, after correcting for baseline, for all groups). As a result, there may have been insufficient ability to detect subtle differences in extinction. The mice were trained in a context with a bright light and underwent extinction trials in the dark. As mice are nocturnal, their activity increases in the dark and freezing behavior to the tone may have been confounded by increased activity simply due to the darker environment. Despite this, mice showed robust between-trial extinction and there was ample opportunity to observe differences between saline and amphetamine-treated mice. To address these concerns, future studies will look at the effect of different conditioning parameters (e.g. increased shock intensity and/or a different number of tone–shock pairings), and extinction training in a bright context.

To conclude, amphetamine does not appear to be a suitable candidate for facilitating fear extinction. As neural mechanisms underlying extinction learning are identified, so are potential targets for pharmacological manipulation. Exposure therapy can successfully be augmented pharmacologically [27], and it would be of significant clinical value to continue searching for those drugs that may enhance extinction. Additionally, to further investigate the dissociation between fear acquisition and extinction learning, it would be useful to concurrently examine acquisition and extinction of fear with a variety of memory-enhancing drugs [9,17,29,35,37].

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## References

[1] P.A. Ahmann, F.W. Theye, R. Berg, A.J. Linquist, A.J. Van Erem, L.R. Campbell, Placebo-controlled evaluation of amphetamine mixture-dextroamphetamine salts and amphetamine salts (Adderall): efficacy rate and side effects, Pediatrics 107 (2001) E10.
[2] S.G. Anagnostaras, G.D. Gale, M.S. Fanselow, Hippocampus and contextual fear conditioning: recent controversies and advances, Hippocampus 11 (2001) 8–17.
[3] S.G. Anagnostaras, S.A. Josselyn, P.W. Frankland, A.J. Silva, Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice, Learn. Mem. 7 (2000) 58–72.
[4] Z. Annau, L.J. Kamin, The conditioned emotional response as a function of intensity of the US, J. Comp. Physiol. Psychol. 54 (1961) 428–432.
[5] M. Barad, P.W. Gean, B. Lutz, The role of the amygdala in the extinction of conditioned fear, Biol. Psychiatry 60 (2006) 322–328.
[6] D.M. Barch, C.S. Carter, W. Perlstein, J. Baird, J.D. Cohen, N. Schooler, Increased stroop facilitation effects in schizophrenia are not due to increased automatic spreading activation, Schizophr. Res. 39 (1999) 51–64.
[7] C.A. Blaiss, P.H. Janak, Post-training, but not post-reactivation, administration of amphetamine and anisomycin modulates Pavlovian conditioned approach, Neurobiol. Learn. Mem. 87 (2007) 644–658.
[8] T.B Borowski, L. Kokkinidis, The effects of cocaine, amphetamine, and the dopamine D1 receptor agonist SKF 38393 on fear extinction as measured with potentiated startle: implications for psychomotor stimulant psychosis, Behav. Neurosci. 112 (1998) 952–965.
[9] J.P. Chhatwal, M. Davis, K.A. Maguschak, K.J. Ressler, Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear, Neuropsychopharmacology 30 (2005) 516–524.
[10] H.J. Dudderidge, J.A. Gray, Joint effects of sodium amylobarbitone and amphetamine sulphate on resistance to extinction of a rewarded running response in the rat, Psychopharmacologia 35 (1974) 365–370.
[11] W.A. Falls, M.J. Miserendino, M. Davis, Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala, J. Neurosci. 12 (1992) 854–863.
[12] M.S. Fanselow, Conditioned and unconditional components of post-shock freezing, Pavlov. J. Biol. Sci. 15 (1980) 177–182.
[13] E.B. Foa, Psychosocial treatment of posttraumatic stress disorder, J. Clin. Psychiatry 5 (Suppl. 61) (2000) 43–48, discussion 49–51.
[14] A. Garakani, S.J. Mathew, D.S. Charney, Neurobiology of anxiety disorders and implications for treatment, Mt. Sinai J. Med. 73 (2006) 941–949.
[15] L. Kokkinidis, The effects of chronic amphetamine administration on the acquisition and extinction of an active and passive avoidance response in mice, Pharmacol. Biochem. Behav. 19 (1983) 593–598.
[16] R. Kumar, Extinction of fear. I. Effects of amylobarbitone and dexamphetamine given separately and in combination on fear and exploratory behaviour in rats, Psychopharmacologia 19 (1971) 163–187.
[17] L. Ledgerwood, R. Richardson, J. Cranney, Effects of D-cycloserine on extinction of conditioned freezing, Behav. Neurosci. 117 (2003) 341–349.
[18] G. Li, S.S. Nair, G.J. Quirk, A biologically realistic network model of acquisition and extinction of conditioned fear associations in lateral amygdala neurons, J. Neurophysiol. 101 (2009) 1629–1646.
[19] K.T. Lu, D.L. Walker, M. Davis, Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle, J. Neurosci. 21 (2001) RC162.
[20] S. Maren, G. Aharonov, D.L. Stote, M.S. Fanselow, N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats, Behav. Neurosci. 110 (1996) 1365–1374.
[21] J.L. Martinez Jr., R.A. Jensen, R.B. Messing, B.J. Vasquez, B. Soumireu-Mourat, D. Geddes, K.C. Liang, J.L. McGaugh, Central and peripheral actions of amphetamine on memory storage, Brain Res. 182 (1980) 157–166.
[22] M.R. Milad, G.J. Quirk, Neurons in medial prefrontal cortex signal memory for fear extinction, Nature 420 (2002) 70–74.
[23] D. Mueller, L.A. Olivera-Figueroa, D.S. Pine, G.J. Quirk, The effects of yohimbine and amphetamine on fear expression and extinction in rats, Psychopharmacology (Berl.) (2009).
[24] K.M. Myers, M. Davis, Mechanisms of fear extinction, Mol. Psychiatry 12 (2007) 120–150.

[25] G.J. Quirk, R. Garcia, F. Gonzalez-Lima, Prefrontal mechanisms in extinction of conditioned fear, Biol. Psychiatry 60 (2006) 337–343.
[26] G.J. Quirk, D. Mueller, Neural mechanisms of extinction learning and retrieval, Neuropsychopharmacology 33 (2008) 56–72.
[27] K.J. Ressler, B.O. Rothbaum, L. Tannenbaum, P. Anderson, K. Graap, E. Zimand, L. Hodges, M. Davis, Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear, Arch. Gen. Psychiatry 61 (2004) 1136–1144.
[28] M.T. Rogan, U.V. Staubli, J.E. LeDoux, Fear conditioning induces associative long-term potentiation in the amygdala, Nature 390 (1997) 604–607.
[29] T. Shuman, S.C. Wood, S.G. Anagnostaras, Modafinil and memory: effects of modafinil on Morris water maze learning and Pavlovian fear conditioning, Behav. Neurosci. 123 (2009) 257–266.
[30] E. Soetens, S. Casaer, R. D'Hooge, J.E. Hueting, Effect of amphetamine on long-term retention of verbal material, Psychopharmacology (Berl.) 119 (1995) 155–162.
[31] E. Soetens, R. D'Hooge, J.E. Hueting, Amphetamine enhances human-memory consolidation, Neurosci. Lett. 161 (1993) 9–12.
[32] A. Suzuki, S.A. Josselyn, P.W. Frankland, S. Masushige, A.J. Silva, S. Kida, Memory reconsolidation and extinction have distinct temporal and biochemical signatures, J. Neurosci. 24 (2004) 4787–4795.
[33] D.C. Turner, T.W. Robbins, L. Clark, A.R. Aron, J. Dowson, B.J. Sahakian, Cognitive enhancing effects of modafinil in healthy volunteers, Psychopharmacology (Berl.) 165 (2003) 260–269.
[34] D.L. Walker, M. Davis, The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction, Pharmacol. Biochem. Behav. 71 (2002) 379–392.
[35] D.L. Walker, K.J. Ressler, K.T. Lu, M. Davis, Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats, J. Neurosci. 22 (2002) 2343–2351.
[36] J.B. Watson, R. Rayner, Conditioned emotional reactions, 1920, Am. Psychol. 55 (2000) 313–317.
[37] S.C. Wood, S.G. Anagnostaras, Memory and psychostimulants: modulation of Pavlovian fear conditioning by amphetamine in C57BL/6 mice, Psychopharmacology (Berl.) 202 (2009) 197–206.

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Chapter 4

# Interdependence of Measures in Pavlovian Conditoned Freezing

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## ABSTRACT

Pavlovian conditioned freezing is an intensively utilized paradigm that has become a standard model of memory and cognition. Despite its widespread use, the interdependence among each measure commonly reported in fear conditioning studies has not been described. Using mice, we examine the relationship of each freezing measure (Training Baseline, Post-Shock freezing, Contextual Fear, Tone Baseline, and Tone Fear), as well as baseline locomotor activity measures, to better understand the significance of each. Of particular interest, Post-Shock freezing appears to be a good measure of immediate contextual memory. In contrast, Tone Baseline freezing, as typically measured in a novel context, appears to be contaminated with multiple sources of fear. Finally, Contextual and Tone Fear show a weak interdependence, reflecting their only partially overlapping neurobiology.


*Keywords*: Pavlovian fear conditioning, classical conditioning, learning and memory, mice

Pavlovian fear conditioning is being used, with increasing frequency, as a measure of learning and memory in a variety of settings, including large-scale pharmacological and genetic screens (Gale *et al.*, 2009; Matynia *et al.*, 2008; Reijmers *et al.*, 2006). The robustness and efficiency of the paradigm has yielded a wealth of findings in recent years, greatly expanding the number of laboratories assessing memory. Fear conditioning uses a rapid procedure that can produce enduring life-long memory (Gale *et al.*, 2004). Training consists of an initially neutral discrete conditioned stimulus (CS), usually a pure tone, paired with an unconditioned stimulus (US), usually a mild footshock. As a result of this pairing, even when presented alone, the CS comes to elicit fear, the conditioned response (CR). Aside from fear of the discrete CS, subjects come to fear the environmental conditions surrounding the fear conditioning episode, a phenomenon known as context conditioning. Contextual fear is measured simply by returning the subject to the training context. Finally, cued fear is assessed by placing the subject in a new context, which is varied on a number of physical dimensions (e.g., appearance, odor, sounds) from the training context, and, after a baseline period to ensure little generalization, playing the tone from the training day (Sanders & Fanselow, 2006).

Both contextual and cued fear are dependent upon the amygdala, whereas contextual fear is further dependent on the hippocampus, in a time-limited fashion (Anagnostaras, Gale, & Fanselow, 2001; Kim & Fanselow, 1992). In contrast, cued fear, as typically performed, is usually found to be hippocampus-independent (e.g., Anagnostaras, Maren, & Fanselow, 1999), but can depend on the hippocampus in

certain conditions, especially when a trace conditioning procedure is utilized (Esclassan, Coutureau, Di Scala, & Marchand, 2009; Maren, 1999; Maren & Holt, 2004; Quinn, Wied, Ma, Tinsley, & Fanselow, 2008). Fear conditioning, therefore, is a well-characterized model of both explicit memory and pathological fear (e.g., specific phobias) in humans (Anagnostaras *et al.*, 2001; Fendt & Fanselow, 1999; Watson & Rayner, 2000).

Freezing, or the lack of movement aside from respiration, is the measure most commonly used to quantify memory in Pavlovian fear conditioning. Freezing is a species-specific defensive reaction, thought to be adaptive for rodents in the face of predators such as snakes and cats (R. C. Bolles, 1970; R.C. Bolles & Riley, 1973). Locomotor activity can also be measured during any of the training or testing measures, but is generally used to assess any sort of movement disturbance due to a genetic or pharmacological manipulation of the subjects being tested, and typically during the baseline periods of the training day, prior to any fear conditioning (Anagnostaras, Josselyn, Frankland, & Silva, 2000; Anagnostaras *et al.*, 2003; Anagnostaras *et al.*, in press; Maren, Aharonov, & Fanselow, 1996).

This paradigm generates several freezing measures. First, Training Baseline is the period during which the animal is initially exposed to the training environment, and is free to explore the box with no tone or shock present. This period is usually at least two minutes because animals may fail to learn contextual cues when the placement-to-shock interval is too short (Fanselow, 1986; Frankland *et al.*, 2004). Training then occurs, with single or multiple presentations of a tone co-terminating

with a footshock. The unconditioned response (UR) to the shock is usually an activity burst, but mice and rats quickly settle into freezing, which Fanselow (1980) has argued is a CR to the context, and not an extended UR as it has sometimes been interpreted (Weiss, Krieckhaus, & Conte, 1968). In either case, freezing during the period of tone-shock pairings or immediately thereafter is known as Post-Shock freezing (PS). Contextual Fear can be examined by measuring freezing when the animal is returned to the training context at a later time, usually one day later. Finally, the tone test, performed an additional day later, is typically divided into the Tone Baseline period, during which the animal explores the novel environment, and a Tone Fear period, during which the animal is exposed to the tone CS, often repeatedly (Anagnostaras *et al.*, 2000; Sanders & Fanselow, 2006).

While these measures are commonly reported, their interdependence (e.g., correlational structure) has not been described. Most significantly, Fanselow has argued that Post-Shock Freezing reflects Contextual Fear (Fanselow, 1980), but it is sometimes still considered a shock UR, or more often, altogether ignored. If Post-Shock freezing is a measure of Contextual Fear, it is an important opportunity to collect the status of short-term memory. Also, while the tone test is designed to measure fear that is independent from Contextual Fear, there can still be much variability in Tone Baseline freezing, especially in mice (Shuman, Wood, & Anagnostaras, 2009; Wiltgen & Silva, 2007; Wiltgen *et al.*, 2010). A recent paper has argued that the standard methods for accounting for differences in the Tone Baseline period (usually subtraction of the baseline from the tone average) is inadequate

(Jacobs, Cushman, & Fanselow, 2010). Context and tone fear can be dissociated in lesion or mutant studies of the hippocampus, but no attention is paid as to whether the two measures are independent in the intact animal (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992).

The current study explores the interdependence of the different measures of a typical fear conditioning study. Because of good power to detect the effects of most independent variables, most studies of fear conditioning use an inadequate sample size to analyze the correlational structure of the dependent measures. Here, a large group of mice were run under the identical fear conditioning protocol, and correlations between the different measures were performed. Moreover, we examined the predictive power of the Post-Shock freezing measure, using a median split. If Post-Shock freezing truly reflects Contextual Fear, as Fanselow (1980) argued, an arbitrary split of Post-Shock freezing should affect only Contextual Fear.

## METHOD

### SUBJECTS

Forty-eight (24 female) Hybrid C57Bl/6Jx129T2SvEms/J (129B6, stock from the Jackson Laboratory, West Sacramento, CA) mice were used. This strain was chosen because it performs in a manner comparable to rats and is widely used in mutant and behavioral studies of fear conditioning (Crawley *et al.*, 1997). Mice were weaned 19 days after birth and were at least 10 weeks old at the time of testing. Mice were group housed (2 to 5 per cage) with unrestricted access to food and water under a

14:10-hr light:dark cycle. Experiments were conducted during the light phase. All animal care and experimental procedures were approved by the University of California, San Diego Institutional Animal Care and Use Committee and were in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals*.

## APPARATUS

### CONDITIONING CONTEXT

Four individual conditioning chambers (Med-Associates, Inc., St. Albans, VT) were located in a windowless room, allowing four mice to be run concurrently. A HEPA air cleaner provided background noise (~65 dBA), and two 100-W bulbs provided bright white light. Each chamber (32 cm wide, 25 cm high, 25 cm deep) was made of three white acrylic sidewalls, and a clear polycarbonate front wall to allow for viewing. Stainless steel drop-pans were scented with 7% isopropyl alcohol to provide background odor and were cleaned between trials. Each chamber contained a stainless steel grid floor (36 rods, each rod 2 mm in diameter, 8 mm center to center), connected to a solid-state scrambler, providing AC constant current shock. A speaker in a sidewall of each chamber was connected to an audio stimulus generator located in an adjacent room. A single color video camera, mounted to the wall facing the conditioning chambers, fed video of the mice to a computer also in the adjacent room. The shock and tone administrations were controlled via an interface connected to a Windows computer running Med-PC (Med-Associates, Inc., St. Albans, VT).

Freezing was scored by a custom-designed software adaptation of NIH Image running on an Apple Macintosh G4 as previously described (Anagnostaras *et al.*, 2000).

ALTERNATE CONTEXT

Four chambers located in a different room than the training context were used to measure tone fear. The chambers (30 cm wide, 25 cm high, 24 cm deep) consisted of solid white walls, floors, and ceilings, with a clear Plexiglas front wall to allow for observation via a wall-mounted infrared video camera, connected to the same computer described above. Each chamber contained a speaker in the sidewall, also connected to the computer. The alternate context was different from the conditioning context along several dimensions: a white acrylic, triangular tent (23 cm each side) formed a tee-pee in each box, the chambers were cleaned with 5% white vinegar between trials, and the room was lit only with dim red light.

PROCEDURE

TRAINING

Training consisted of a 2 min baseline period (Train.BL), followed by one tone-shock pairing. The tone was 30 s (2.8 kHz, 85 dBA), co-terminating with a scrambled, constant current AC footshock (2 s, 0.75 mA, RMS). Immediate post-shock freezing was measured for another 2.5 min (PS), resulting in a 5 min, total, exposure to the training context.

TESTING

Contextual Fear (Context) was measured by returning the mice to the conditioning context 24 h after training for 5 min. Tone (cued) fear was measured 48 h after

training, in the alternate context. Testing consisted of a 2 min baseline period (Tone.BL), followed by a 3 min tone identical to the tone used in training (Tone). Freezing was used as the dependent measure for both tests.

## RESULTS

### TRAINING AND TESTING

Figure 1 shows the group means for fear conditioning, typical for the paradigm. Freezing increased from the training baseline period, Train.BL (average, 12.3±0.8%) to the post-shock period, PS [29.0±1.6%; Fig 1A; ANOVA, $F(1, 47)=81.6$, $p<0.0001$]. Mice also showed high levels of freezing when returned to the training context 24 h later, Context (average, 47.6±2.1%, Fig 1B). Finally, subjects displayed little freezing when initially introduced to the alternate context, during Tone.BL (average, 5.9±0.8%), but demonstrated significantly higher levels of freezing when the tone was presented, Tone [45.3±2.7%, Fig 1C; $F(1, 47)=230.1$, $p<0.0001$].

### POST-SHOCK FREEZING MEDIAN SPLIT

To explore the relationship of post-shock freezing to other measures, subjects were split into two groups, based upon their performance during PS (min 4 and 5 of training; see Fig 2). Mice in the "High PS" ($n = 23$) group froze during 27% of PS or more, while the "Low PS" ($n = 25$) group froze less than 27% of PS. Remarkable selectivity in the effect of this median split was found. Mice in the two groups showed no difference in baseline freezing (min 1 and 2) during Train.BL [$F(1, 46)=0.01$, *n.s.*], but froze at significantly different levels during PS [Fig 2A; $F(1, 46)=73.2$, $p<0.0001$]. Most significantly, the "High PS" group froze more than the

"Low PS" group during Context [Fig 2B; $F(1, 46)=11.3$, $p<0.01$]. Moreover, no difference was seen between the two groups during either Tone.BL [$F(1, 46)=0.07$, *n.s.*] or during Tone [Fig 2C; $F(1, 46)=0.33$, *n.s.*]. Finally, differences in Post-Shock Freezing were in no way attributable to differences in activity (automatically measured by the computer software in arbitraty units, au; Anagnostaras *et al.*, 2000) as there were no differences found in terms of locomotor activity during either the training [A.Train.BL; $F(1, 46)=0.39$, *n.s.*] or tone baseline [A.Tone.BL; $F(1, 46)=1.26$, *n.s.*] periods, as well (Fig 2D). Thus, the median split on Post-Shock freezing showed remarkable selectivity for Contextual Fear, indicating it is indeed a measure of Contextual Fear. This is in agreement with Fanselow (1980), who, using an entirely different approach, reached the same conclusion (see Discussion).

POST-SHOCK FREEZING CORRELATIONS

The interdependence of the measures was examined first by correlating PS with the other freezing measures. PS freezing was highly correlated with freezing during Context (Fisher's r-to-z; $r=0.65$, $p<0.0001$), but was largely unrelated to Tone ($r=0.23$, *n.s.*), Tone.BL ($r=0.09$, *n.s.*), or Train.BL ($r=-0.04$, *n.s.*) freezing (Fig 3A-D).

OVERALL CORRELATIONS - FREEZING

Figure 4A depicts the correlations among all periods of freezing during testing and training, in descending strength of their *r* values of each correlation. As mentioned above, PS freezing was highly correlated with Context ($r=0.65$, $p<0.0001$). Freezing during Context was also weakly correlated with freezing during Tone.BL ($r=0.34$, $p<0.05$), as well as with freezing during Tone ($r=0.29$, $p=0.05$). Tone.BL and Tone

freezing were also correlated ($r$=0.31, $p$<0.05). Overall, the only strong correlation was between Post-Shock freezing and Contextual Fear. However, other measures of freezing (except for Train.BL) were also weakly correlated. One must keep in mind that these share a common neurobiology (e.g., the amygdala and periaqueductal gray matter; Fendt & Fanselow, 1999; Kim, Rison, & Fanselow, 1993), and are thought to be mediated by the same shock memory (Rescorla, 1974). The only disturbing finding is that Tone.BL did not entirely reflect generalized Contextual Fear as most investigators, including us, would implicitly assume. Tone Baseline freezing was a highly contaminated measure, correlated with both Contextual and Tone Fear (see also Jacobs *et al.*, 2010).

OVERALL CORRELATIONS – BASELINE ACTIVITY

Periods of locomotor activity during the training and tone test baselines were compared to all freezing measures in Fig 4B. The activity during tone baseline (A.Tone.BL) was highly negatively correlated with Tone.BL freezing ($r$=-0.68, $p$<0.0001). While this may seem like a necessary relationship, it is worth noting that activity and freezing during Train.BL were not significantly correlated ($r$=–0.21, *n.s,*), and Train.BL measures were not significantly related to any other measures. This suggests that even though there was more overall activity in the Tone.BL period [as it was performed in the dark; $F(1,47) = 31.5$, $p < 0.0001$; Fig 2D], the activity during the Train.BL is less dominated by a fear reaction as it occurs prior to any shock. Activity during Tone.BL was also correlated negatively with Tone freezing ($r$=–0.40, $p$<.01) and Context freezing ($r$=–0.39, $p$<.01), reflecting similar contamination to Tone.BL

freezing. Finally, activity during Train.BL and Tone.BL were unrelated ($r$=-0.03, *n.s.*).

## DISCUSSION

The findings presented above provide researchers today with a better understanding of the importance and meaning of each fear conditioning measure commonly reported. Most significantly, Post-shock freezing is clearly short-term memory for contextual fear. Fanselow (1980) reached this same conclusion using an entirely different approach. It is well known that when the placement-to-shock interval is very short (e.g., 5 sec) animals fail to acquire contextual fear, as measured on a typical test one day later, a phenomenon known as the "immediate shock deficit" (Fanselow, 1986; Frankland *et al.*, 2004). What is less known is that those same animals show a normal activity burst UR, but fail to show post-shock freezing. Moreover, Fanselow (1980) also found that post-shock freezing was eliminated if animals were moved to a different context immediately following the shock. Taken together, these data suggest that Post-Shock freezing can and should be used as a measure of "immediate," or "short-term memory" for contextual fear (see, also, discussion in Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999). We provide the caveat, however, that when utilizing fear conditioning for pharmacological studies, care in interpreting the results must be taken if the drug leads to altered levels of activity while administered for the training period (Wood & Anagnostaras, 2009; Wood, Fay, Sage, & Anagnostaras, 2007).

Contextual and tone fear are weakly correlated, as well, which is not altogether surprising. While the two fear measures are generally treated as dissociable, both types of memory share substantial neurobiology (e.g., the amygdala, periaqueductal grey matter, NMDA receptors, etc.) as well as a common motivational component (Fanselow & Gale, 2003; Fendt & Fanselow, 1999; Kim *et al.*, 1993; Rabinak, Orsini, Zimmerman, & Maren, 2009; Rescorla, 1974). It is not hard to imagine that, in intact animals, some may have perceived the shock as more painful, leading to higher freezing scores in both conditions.

We also found that the tone baseline period is a problematic area for the standard paradigm. Although rats typically do not usually show freezing during this period, and it does not increase with the passage of many days (e.g., Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992), mice often show some freezing during this period that can even increase with the passage of weeks (Wiltgen & Silva, 2007). Unfortunately, we found that Tone Baseline freezing is equally correlated with both Contextual Fear and Tone Fear, and thus does not purely reflect contextual generalization. Moreover, locomotor activity during the Tone Baseline is negatively correlated with both Contextual and Tone Fear. In contrast, Activity during the Training Baseline was unrelated to any other measure, including activity during the tone baseline. Overall, these findings suggest that subtracting tone baseline freezing from tone freezing, as is often performed, is problematic. Jacobs et al. (2010), by manipulating extinction and training to the two contexts, reached the same conclusion, and further found that the relationship between tone baseline and tone fear is not

strictly linear. They suggest, and we concur, that the greatest effort must be made to eliminate Tone Baseline freezing. The approach they suggest is a bit convoluted, utilizing multiple days of extinction of the training and/or alternate context prior to tone testing, to ensure a low baseline in the alternate context. It remains to be seen if this approach is practical, or if the resulting low tone baseline freezing will be long-lasting in mice. We further suggest it may be possible to solve this problem in mice by further enhancing the differences between the two contexts. For example, one might not use a Skinner-type chamber at all, such as modified home cage or something altogether different for the alternate context (see for e.g., Anagnostaras & Robinson, 1996) or simplifying the design of Jacobs et al., extinguishing only the alternate context prior to tone testing.

Overall, these findings help of the interpretation of Pavlovian fear conditioning data being produced today in increasing numbers. The Post-Shock period of the training day is a good indication of short-term contextual fear memory, because it is correlated strongly only with contextual fear measured later. Fear exhibited on the tone test day is more difficult to interpret, with generalization from the Context Test accounting for only some, but not all of the fear exhibited during the baseline period. It is tempting to standardize the Pavlovian conditioned freezing procedure as it develops into a standard assay of memory in various screening settings (Maren, 2008). Despite the robustness of the paradigm, the data suggest some further improvement could occur in the area of discrete cue testing (Jacobs *et al.*, 2010).

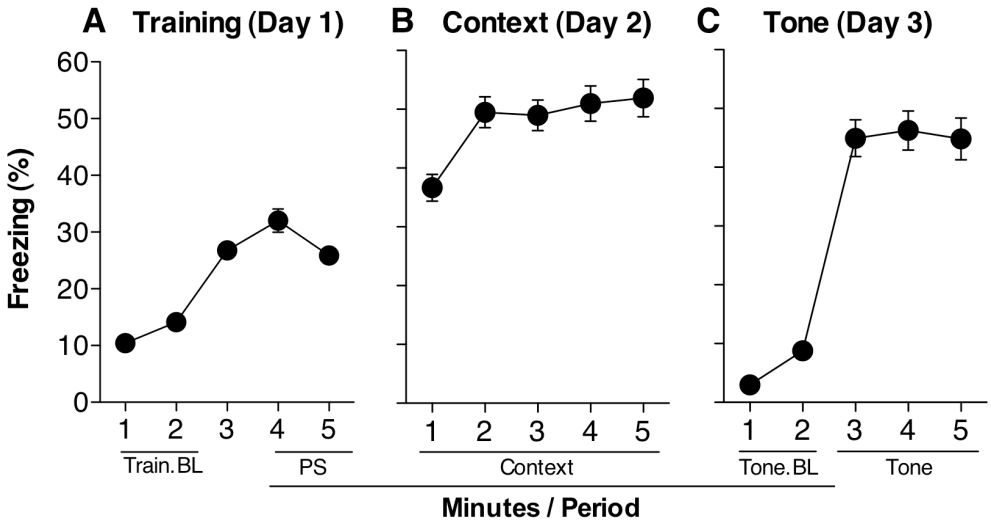


**Figure 1**

*Freezing during training and testing.* Percent time spent freezing (±SEM) is presented in one min bins for the training day, context test, and tone test. Training (A) is made up of the baseline period, during which no tone or shock is presented (Train.BL), as well as a post-shock period, a measure of freezing immediately following the tone-shock presentation (PS). The subject is returned to the training context 24 h later for the context test (B), during which freezing to the context is measured for 5 min (Context). The tone test (C) consists of a tone baseline period (Tone.BL), during which the animal is free to explore the novel environment, and the tone test (Tone), when the tone identical to that played on the training day is presented.

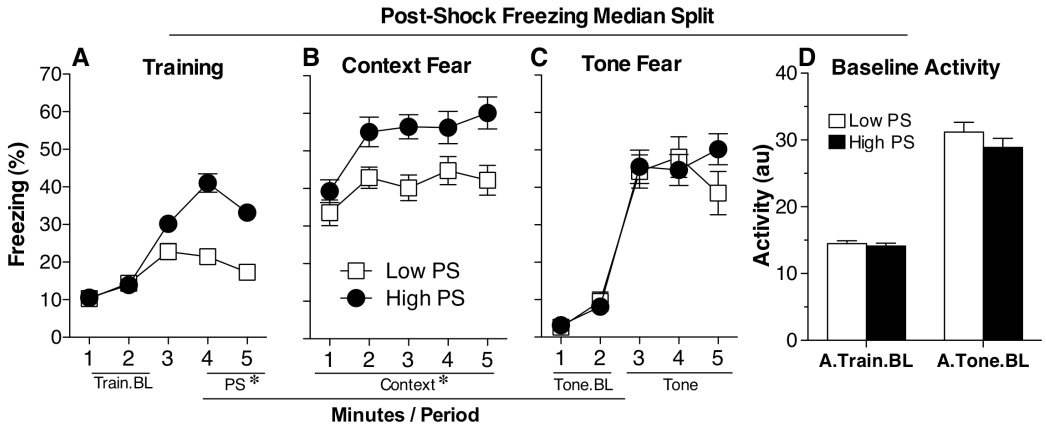


**Figure 2**

*Post-shock freezing median split.* Subjects were divided into two groups based on their fear during the PS period of training. This division created two statistically significantly different groups for the PS period (A; * indicates $p < .05$), but not for the Train.BL period. Likewise, the two groups continued to display fear at significantly different levels during the Context Test (B), but not the Tone.BL or Tone periods of the tone test (C). The two groups also showed no difference in baseline activity, either during training or the tone test (D).

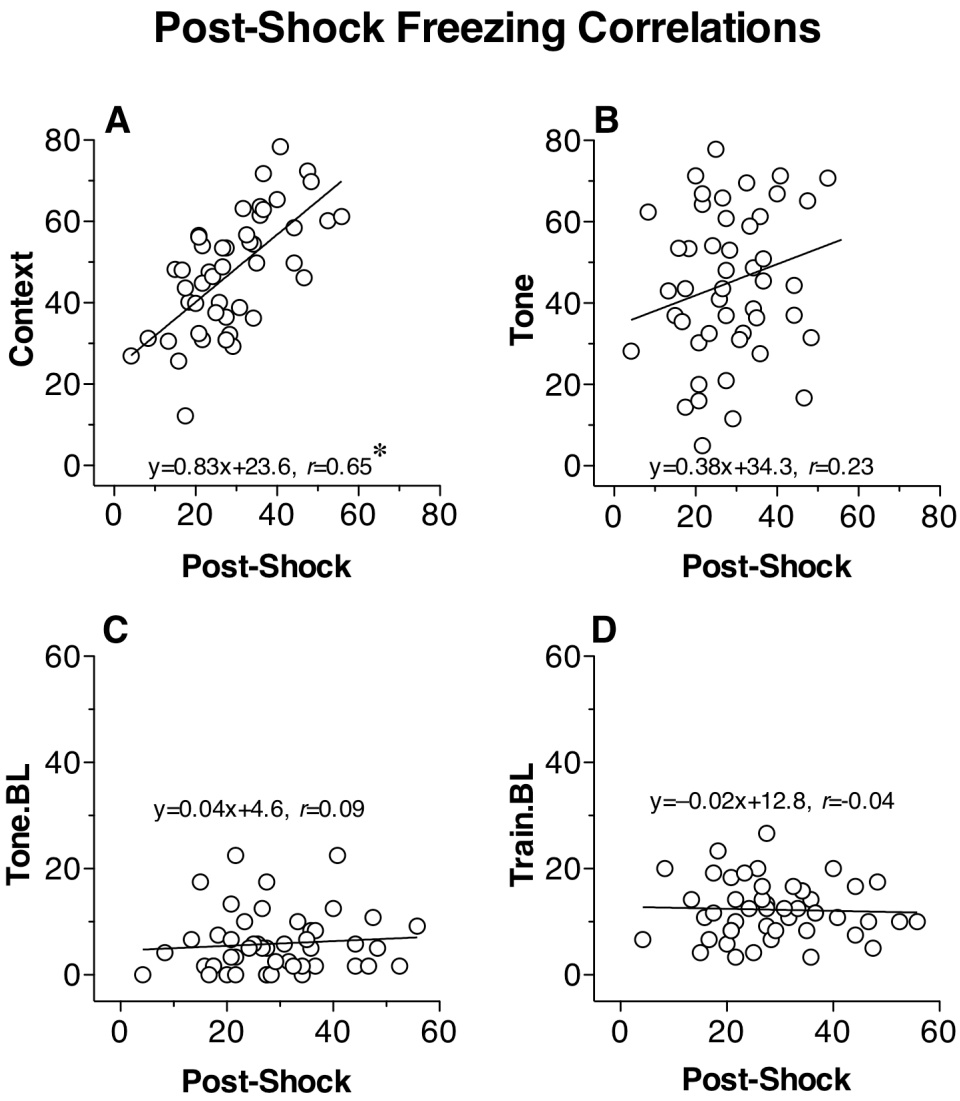


**Figure 3**
*Post-shock freezing correlations.* Post-shock freezing was significantly correlated with context fear (A), but not tone (B), tone baseline (C) or training baseline (D).

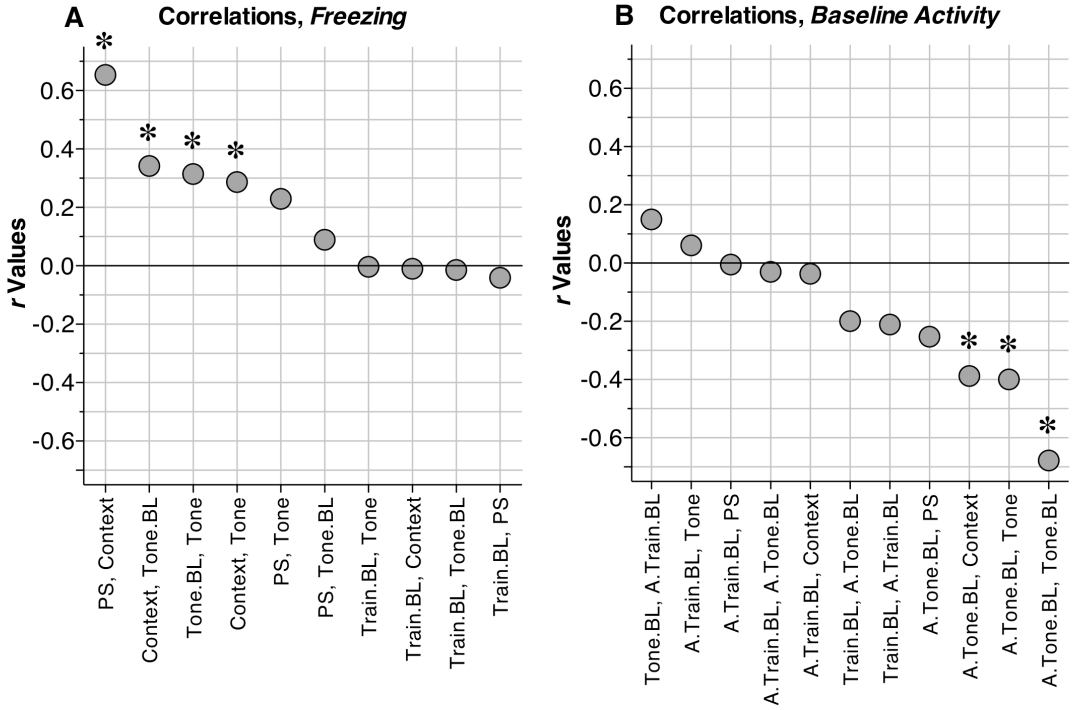


**Figure 4**

*Correlations.* Correlations among all the freezing measures are presented (A), with PS-Context, Context-Tone.BL, Tone.BL-Tone, and Context-Tone all showing significant, positive correlations. Correlations among baseline activity measures and freezing measures are also shown (B). The strongest relationship was found between the A.Tone.BL (A. designating measures of activity) and Tone.BL, with A.Tone.BL having significant correlations with both Tone and Context freezing measures.

REFERENCES

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus, 11*(1), 8-17.

Anagnostaras, S. G., Josselyn, S. A., Frankland, P. W., & Silva, A. J. (2000). Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. *Learn Mem, 7*(1), 58-72.

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci, 19*(3), 1106-1114.

Anagnostaras, S. G., Maren, S., Sage, J. R., Goodrich, S., & Fanselow, M. S. (1999). Scopolamine and Pavlovian fear conditioning in rats: dose-effect analysis. *Neuropsychopharmacology, 21*(6), 731-744.

Anagnostaras, S. G., Murphy, G. G., Hamilton, S. E., Mitchell, S. L., Rahnama, N. P., Nathanson, N. M., & Silva, A. J. (2003). Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci, 6*(1), 51-58.

Anagnostaras, S. G., & Robinson, T. E. (1996). Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behav Neurosci, 110*(6), 1397-1414.

Anagnostaras, S. G., Wood, S. C., Shuman, T., Cai, D. J., LeDuc, A. D., Zurn, K. R., Zurn, J. B., Sage, J. R., & Herrera, G. M. (in press). Automated assessment of Pavlovian conditioned freezing and shock reactivity in mice using the VideoFreeze system. *Frontiers in Behavioral Neuroscience*.

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review, 77*(1), 32-48.

Bolles, R. C., & Riley, A. L. (1973). Freezing as an avoidance response: Another look at the operant-respondent distinction. *Learning and Motivation, 4*(3), 268-275.

Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A. J., Wehner, J. M., Wynshaw-Boris, A., & Paylor, R. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl), 132*(2), 107-124.

Esclassan, F., Coutureau, E., Di Scala, G., & Marchand, A. R. (2009). Differential

contribution of dorsal and ventral hippocampus to trace and delay fear conditioning. *Hippocampus, 19*(1), 33-44.

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci, 15*(4), 177-182.

Fanselow, M. S. (1986). Associative vs Topographical Accounts of the Immediate Shock-Freezing Deficit in Rats: Implications for the Response Selection Rules Governing Species-Specific Defensive Reactions. *Learning and Motivation, 17*, 16-39.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev, 23*(5), 743-760.

Frankland, P. W., Josselyn, S. A., Anagnostaras, S. G., Kogan, J. H., Takahashi, E., & Silva, A. J. (2004). Consolidation of CS and US representations in associative fear conditioning. *Hippocampus, 14*(5), 557-569.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., Wiltgen, B., & Fanselow, M. S. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Gale, G. D., Yazdi, R. D., Khan, A. H., Lusis, A. J., Davis, R. C., & Smith, D. J. (2009). A genome-wide panel of congenic mice reveals widespread epistasis of behavior quantitative trait loci. *Mol Psychiatry, 14*(6), 631-645.

Jacobs, N. S., Cushman, J. D., & Fanselow, M. S. (2010). The accurate measurement of fear memory in Pavlovian conditioning: Resolving the baseline issue. *J Neurosci Methods, 190*(2), 235-239.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behav Neurosci, 107*(6), 1093-1098.

Maren, S. (1999). Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats. *Behav Neurosci, 113*(2), 283-290.

Maren, S. (2008). Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. *Eur J Neurosci, 28*(8), 1661-1666.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. *Behav Neurosci, 110*(4), 718-726.

Maren, S., & Holt, W. G. (2004). Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. *Behav Neurosci, 118*(1), 97-110.

Matynia, A., Anagnostaras, S. G., Wiltgen, B. J., Lacuesta, M., Fanselow, M. S., & Silva, A. J. (2008). A high through-put reverse genetic screen identifies two genes involved in remote memory in mice. *PLoS One, 3*(5), e2121.

Quinn, J. J., Wied, H. M., Ma, Q. D., Tinsley, M. R., & Fanselow, M. S. (2008). Dorsal hippocampus involvement in delay fear conditioning depends upon the strength of the tone-footshock association. *Hippocampus, 18*(7), 640-654.

Rabinak, C. A., Orsini, C. A., Zimmerman, J. M., & Maren, S. (2009). The amygdala is not necessary for unconditioned stimulus inflation after Pavlovian fear conditioning in rats. *Learn Mem, 16*(10), 645-654.

Reijmers, L. G., Coats, J. K., Pletcher, M. T., Wiltshire, T., Tarantino, L. M., & Mayford, M. (2006). A mutant mouse with a highly specific contextual fear-conditioning deficit found in an N-ethyl-N-nitrosourea (ENU) mutagenesis screen. *Learn Mem, 13*(2), 143-149.

Rescorla, R. A. (1974). Effect of inflation of the unconditioned stimulus value following conditioning. *J Comp Physiol Psychol, 86*(1), 101-106.

Sanders, M. J., & Fanselow, M. S. (2006). The Behavioral Neuroscience of Pavlovian Fear Conditioning. In M. J. Anderson (Ed.), *Tasks and Techniques: A Sampling of the Methodologies for the Investigation of Animal Learning, Behavior and Cognition*. Hauppauge, NY: Nova Science Publishers, Inc.

Shuman, T., Wood, S. C., & Anagnostaras, S. G. (2009). Modafinil and memory: effects of modafinil on Morris water maze learning and Pavlovian fear conditioning. *Behav Neurosci, 123*(2), 257-266.

Watson, J. B., & Rayner, R. (2000). Conditioned emotional reactions. 1920. *Am Psychol, 55*(3), 313-317.

Weiss, J. M., Krieckhaus, E. E., & Conte, R. (1968). Effects of fear conditioning on subsequent avoidance behavior and movement. *J Comp Physiol Psychol, 65*(3), 413-421.

Wiltgen, B. J., & Silva, A. J. (2007). Memory for context becomes less specific with time. *Learn Mem, 14*(4), 313-317.

Wiltgen, B. J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., Li, W., & Silva, A. J. (2010). The hippocampus plays a selective role in the retrieval of detailed contextual memories. *Curr Biol, 20*(15), 1336-1344.

Wood, S. C., & Anagnostaras, S. G. (2009). Memory and psychostimulants: modulation of Pavlovian fear conditioning by amphetamine in C57BL/6 mice. *Psychopharmacology (Berl), 202*(1-3), 197-206.

Wood, S. C., Fay, J., Sage, J. R., & Anagnostaras, S. G. (2007). Cocaine and Pavlovian fear conditioning: dose-effect analysis. *Behav Brain Res, 176*(2), 244-250.

Chapter 4, in full, has been submitted for publication of the material as it may appear in Interdependence of Measures in Pavlovian Conditioned Freezing in *Behavioral Neuroscience*. Wood, S.C. & Anagnostaras, S.G. (2010). The dissertation author was the primary investigator and author of this material.

# Chapter 5

## Psychostimulants and cognition: a continuum of activation

Psychostimulants are a broad class of sympathomimetic drugs whose effects can increase movement, arousal, vigilance, vigor, wakefulness, and attention (Westfall and Westfall, 2006). Some psychostimulants, especially at high doses and with a rapid route of administration, produce euphoria, a sense of power and confidence, and addiction in susceptible individuals. The present review focuses on the cognitive effects of psychostimulants, with particular attention to low doses associated with cognitive enhancement (Arnsten, 2006; Kuczenski and Segal, 2002; Wood and Anagnostaras, 2009). Although some traditional definitions of the term *psychostimulant* require that the drug increase locomotion in rodents, here we argue that the term *psychostimulants* should include drugs that may not produce hyperlocomotion (e.g., caffeine and modafinil). We refer to the more narrow class of psychostimulants that also produce significant hyperlocomotion (e.g., cocaine and amphetamine) as *psychomotor stimulants*. Moreover, even psychomotor stimulants only do so for a certain range of doses (see Wood *et al*, 2009; Wood *et al*, 2007).

Psychostimulants, broadly construed, include drugs of abuse, such as cocaine and methamphetamine, as well as therapeutic drugs such as amphetamine salts (Adderall, Vyvanse), methylphenidate (Concerta, Focalin), and modafinil (Provigil). Psychostimulants are also used nonmedically, with caffeine, coca leaves, and khat being examples of stimulants consumed today for primarily quality of life purposes.

Casual use of stimulants for wakefulness or performance enhancement dates back centuries. For example, evidence for medical use of khat (which contains cathinone, a low potency amphetamine-like stimulant), popular in parts of the Middle East and Africa, dates back to at least the 11th century (Al-Motarreb *et al*, 2002). Today, khat is a social mainstay in several countries (e.g., Yemen), and an important source of financial revenue in others (e.g., Ethiopia; Al-Hebshi and Skaug, 2005). Interestingly, and in parallel with Western medicine's approach towards improving academic performance in children with attention deficit/hyperactivity disorder (ADHD), khat is sometimes given to school-aged children by parents who believe that it improves studying (Al-Motarreb *et al*, 2002).

In the United States and other Western cultures, it is not khat, but amphetamine and other closely related drugs that are successfully used in the treatment of a variety of disorders, including ADHD. However, psychostimulants are also subject to abuse that can sometimes lead to addiction. Although addiction is thought of as an interaction between certain susceptible traits and particular addictive drugs, the difference between performance enhancement and addiction, with respect to stimulants, prominently depends on two closely related factors: dose and route of administration. These two factors are integral to the problem of addiction, a disorder that is hypothesized to hijack healthy learning and memory circuits, altering them to focus almost solely on the procurement and consumption of the drug of abuse (Hyman, 2005).

We begin this review with an overview of several popular psychostimulants: cocaine, amphetamine, methylphenidate, modafinil, and caffeine. We then propose a ***continuum of psychostimulant activation***, outlining the full range of responses typically seen after psychostimulant administration, with low doses producing beneficial cognitive effects and high doses producing addiction and psychosis. Cognitive and performance enhancement will be closely associated with low doses, less efficacious drugs, and slow (usually oral) administration, whereas addiction will be closely associated with high doses, more efficacious or potent drugs, and rapid routes of administration (insufflation or injection; Bickel *et al*, 2007).

## COCAINE

### HISTORY OF USE

Cocaine is an alkaloid derived from the coca plant, typically extracted in a paste form and converted into a salt because of the instability of the free base. This salt can be prepared in a variety of ways to facilitate intake by methods such as i.v. injection or snorting, or converted back to a free base for smoking (i.e., "crack"). However, the coca plant has been used for centuries in Central and South America for its stimulant effects. In Chile, cocaine metabolites can be detected in mummified human remains dating back 2,000 years (Cartmell *et al*, 1991). Dental examination of remains in Chile and Peru suggest a prevalence of coca chewing close to 40% in the prehistoric populations (Indriati and Buikstra, 2001). It is also claimed that no profound illness is found in studies of modern habitual chewers of coca leaf. This

may be attributable to the low dose used by habitual chewers, in contrast to the high doses given in laboratory studies or taken by addicts (Hanna, 1974). Bolivia considers the coca leaf and cocaine to be very separate issues, to the degree that President Evo Morales is known for declaring "Long live the coca leaf, death to the Yankees" in response to U.S. pressure to curb coca leaf production (Schipani, 2008).

In the West, cocaine was widely used toward the latter half of the 19th century in coca wines, cigarettes, and patent medicines, including Coca-Cola. The beneficial effects of cocaine were famously expounded on by Sigmund Freud (who used low doses) in his essay, *Über Coca* (Shaffer, 1984): "exhilaration and lasting euphoria, which in no way differs from the normal euphoria of the healthy person...You perceive an increase of self-control and possess more vitality and capacity for work...Long intensive physical work is performed without any fatigue." Cocaine addiction was recognized by the late 1880s (Mattison, 1887), but cocaine use was not illicit in most countries including the United States until 1914, following the International Opium Convention of 1912 (the first of many international treaties regulating drug abuse).

MECHANISM OF ACTION

Cocaine blocks the reuptake of monoamine neurotransmitters, including dopamine (DA) norepinephrine (NE) and serotonin (5-HT). Blockade of DA reuptake has been closely associated with the reinforcing and addictive properties of cocaine (O'Brien, 2006). Furthermore, behavioral studies have shown reduced striatal dopaminergic functioning in recreational cocaine users (Colzato *et al*, 2008), while PET studies have confirmed a reduction in dopamine $D_2$ receptor availability in

cocaine abusers, even after months of abstinence (Volkow *et al*, 1993). Cocaine's behavioral activating and dopaminergic effects further depend on glutamate N-Methyl-D-Aspartate (NMDA) receptors, although the exact mechanism is somewhat unclear. NMDA receptors are functionally coupled to D2 receptors in the striatum, and this coupling is critical for even the acute behavioral response to cocaine (Liu *et al*, 2006). Blockade of NMDA receptors reduces the efficacy of cocaine in terms of its ability to increase extracellular dopamine concentrations, and reduces cocaine's locomotor activating effects. Finally, activation of DA triggers second messengers that regulate expression of both NMDA and AMPA receptors (Svenningsson *et al*, 2005).

Cocaine also blocks sodium channels, thereby acting as a powerful local anesthetic (Billman, 1990; Rump *et al*, 1995), its only medically approved use in the U.S. This mechanism is not shared with other stimulants such as amphetamine, and has been blamed for cocaine's cardiotoxic and convulsigenic effects. However, recent evidence suggests that cocaine's convulsigenic effects may be due to action at NMDA receptors (see Lason, 2001 for a brief review).

Outside of Freud's (1884) description, there is little formal knowledge regarding the potential performance enhancing effects of cocaine, which might occur at lower doses than those used by addicts, because cocaine is not prescribed for those purposes.

## THERAPEUTIC USE

According to the Drug Enforcement Administration (DEA) of the United States, cocaine is a Schedule II drug, considered to be highly addictive and dangerous to the user's health, but appropriate for medicinal use (DEA, 2002). Both prolonged and acute use of cocaine can lead to a wealth of cardiotoxic effects, the severity of which is dependent upon the dose used (Kloner *et al*, 1992). However, cocaine inhibits sodium ($Na^+$) channels at high concentrations, and historically was widely used as a local anesthetic especially in dentistry and opthamology (Catterall and Mackie, 2006). Today, similar compounds (e.g., lidocaine) are more commonly used for this purpose. Medicinal cocaine use is rare, but sometimes argued to be superior for certain eye or ear surgeries because it has combined vasoconstrictive properties in addition to local anesthesia (Catterall *et al*, 2006; Henderer and Rapuano, 2006).

## ABUSE

The effects of different doses of cocaine on human cognition are difficult to pinpoint. While drugs such as caffeine can ethically be administered within a range of doses to undergraduate populations, cocaine can only be administered exclusively to those who have recently or are currently using the drug (an estimated 2.1 million people in 2007, or 0.8 percent of the U.S. population, according to a recent survey by the Substance Abuse and Mental Health Services Administration, SAMHSA, 2008). Caffeine intake can be estimated with a reasonable level of accuracy by the number of cups of coffee, soda, or other caffeinated beverages consumed per day. The consumption of cocaine or any illegal, highly addictive substance, on the other hand,

is difficult to estimate when bought on the street, as it may be laced with any number of other substances. If attempting to estimate the amount of cocaine consumed by users, researchers have done so by the self-reported amount of money spent on the drug per week (Bolla *et al*, 2003), amount consumed in a month's time (Colzato *et al*, 2008), or within a week (Goldstein *et al*, 2004).

There is abundant evidence that habitual cocaine use impairs a host of neurocognitive functions. As calculated by a recent analysis, cocaine's largest impact on cognition is evidenced by tests of attention, as well as visual and working memory (Jovanovski *et al*, 2005). An electrophysiological study of cocaine dependent participants revealed a reduced amplitude in the P300 component, considered to reflect an impairment in attention and working memory functions, compared to controls (Gooding *et al*, 2008). Likewise, a study of 42 crack-cocaine-addicted individuals demonstrated a general, mild level of cognitive impairment as measured by a battery of 16 different tests (Goldstein *et al*, 2004).

Disruption of prefrontal cortical activation has also been found in cocaine abusers. A recent study showed that cocaine users, defined here as those who self-administer cocaine at least four times per month, performed as well as controls on the Stroop Task (Bolla *et al*, 2004). Positron emission tomography (PET) images revealed, however, that the cocaine users showed less activation in the left anterior cingulate cortex (ACC) and right lateral prefrontal cortex (LPFC) compared to controls, while exhibiting higher levels of activation in the right ACC. Moreover, the greater amount of self-administered cocaine per week leading up to the 23 days of

enforced abstinence, the lower the activity in the rostral ACC and the right LPFC. Similarly, a PET study from the same research group revealed an increased activation in the right orbitofrontal cortex (OFC) and decreased activation in the right dorsolateral prefrontal cortex (DLPFC) in cocaine abusers, as compared to controls, while performing the Iowa Gambling Task, a challenging task of executive function that lesion studies have shown is related to OFC function (Bolla *et al*, 2003). In this study, as well, the amount of cocaine consumed prior to the enforced abstinence period was negatively correlated with left OFC activation. Metabolism in the DLPFC was also found to predict performance on tasks tapping into visual memory and verbal memory (Goldstein *et al*, 2004). These studies indicate that frontal areas involved in attention and executive functioning are particularly hard-hit by extended cocaine use, although compensatory mechanisms may be in place to assist in performing simple attention tasks such as the Stroop Test.

Other studies have shown a mixture of impairments as well as enhancements on different cognitive tasks in cocaine dependent individuals. Thirty-eight crack cocaine dependent men showed a host of deficits compared to controls, including poorer performance in object-naming ability as measured by the Boston Naming Test, executive control as measured by the Booklet Categories Test, spatial memory as measured by the Benton Visual Retention Test, and concentration or speed as measured by Trails B. Interestingly, cocaine dependent participants performed better on the Controlled Oral Word Association Test, a word-list generation task that measures verbal fluency. They also achieved a higher number of correct categories on

the Wisconsin Card Sorting Test (WCST), thought to measure executive functioning. Performance on these cognitive tasks did not show a relationship to years of cocaine abuse or abstinence, making the implications of these results difficult to interpret (Hoff *et al*, 1996). These findings are supported by other research, however, with 30 polysubstance abusers, 28 of whom used cocaine regularly for an average of over seven years, performing as well as controls on the WCST (Grant *et al*, 2000). In this study, drug abusers showed a marked deficit on performance of the more challenging Gambling Task, which also tests executive function.

SUMMARY

> *I snorted the first line and initially didn't feel much…Gradually, I became aware that my mood was significantly elated. I had another line and…I seemed to have much quicker and more incisive analytical abilities. After the next line…I felt like a God. I felt untouchable, invincible.*[1]

While the studies reviewed above demonstrate clearly that prolonged use of high-dose cocaine leads to a variety of cognitive impairments, virtually no studies have examined the effects of low-dose cocaine. Our lab examined the effects of a wide range of doses of cocaine (0.1 – 15 mg/kg, i.p.) on a simple learning paradigm, fear conditioning in rodents (Wood *et al*, 2007). We found that while a moderately high dose of cocaine (15 mg/kg, i.p.), similar to what addicts might take, led to memory impairments, while a low dose (0.1 mg/kg, i.p.) actually enhanced memory (Figure 2). Cocaine also produced hyperlocomotion at even the lowest doses (Figure 1). This is an indication that cocaine could work in a similar fashion to the rest of the

[1] http://www.drugs-forum.com/forum/showthread.php?t=9078&highlight=Cocaine+Experiences (retrieved on 9/6/2010)

stimulants discussed, herein. Low doses may lead to cognitive enhancements, while high doses lead to cognitive impairments, and in this case, addiction.

## AMPHETAMINE

### HISTORY OF USE

Use of amphetamine and similar compounds been documented for centuries. Ephedra (also known as ma huang), specifically the *Ephedra sinica* species, is an herb that has been used in Traditional Chinese Medicine (TCM) for thousands of years (Abourashed *et al*, 2003). While used in TCM primarily for the treatment of asthmatic symptoms, modern use in the U.S. of ephedra and its active ingredient, ephedrine, has revolved around weight loss and performance enhancement (Mehendale *et al*, 2004). After mounting evidence for their involvement in adverse side effects and death, the U.S. Food and Drug Administration (FDA) banned the sale of dietary supplements containing ephedra in 2004 (FDA, 2004). Ephedrine, however, remains for sale in certain antiasthmatics.

The efficacy of ephedra as a bronchodilator encouraged the medical community to seek out a synthetic, inexpensive version of the herbal remedy in the early 1900s (Meyer & Quenzer, 2005). Amphetamine was marketed as a nasal inhaler under the brand name Benzedrine (mixed D and L-amphetamine salts). Benzedrine was also administered in pill form, used to treat maladies including sea sickness, narcolepsy, and Parkinson's Disease (Davies *et al*, 1939). The U.S. military showed an interest in Benzedrine, although one study found that it provided little to no

alleviation of physical and mental fatigue in U.S. soldiers (Somerville, 1946). Interestingly, another study found that Benzedrine led to improved school performance in roughly half of the child participants, who were at a hospital due to a range of behavior disorders (Bradley, 1937). While this is some of the early evidence demonstrating the cognitive enhancing abilities of stimulants, another report also provides early evidence for academic doping (see discussion of academic doping in Abuse-Academic Doping within the Methylphenidate section). Severe cardiac collapse occurred after excessive Benzedrine had been self-administered by one individual who "said the drug was being used to some extent by persons studying for examinations" (Davies *et al*, 1939). Dexedrine (pure D-amphetamine) was introduced as a more potent version of the drug, and, in 1944, Methamphetamine (Methedrine) was introduced as the most potent amphetamine, prescribed for hay fever, alcoholism, narcolepsy, and other indications (FDA, 2010). Today it remains approved only for ADHD and obesity (Ovation Pharmaceuticals, 2007).

MECHANISM OF ACTION

Amphetamine acts to dramatically increase the amount of extracellular monoamines available in the brain, through reversal of the DA, NE, and 5HT reuptake transporters and regulation of their surface expression levels. Converging evidence suggests that amphetamine enters the cell through various monoamine reuptake transporters, and reverses the vesicular monoamine transporter. This leads to a large release of cytoplasmic and vesicular stores of transmitter (Robertson *et al*, 2009). In contrast to cocaine, the release of transmitter is $Ca^{2+}$ independent, (Sulzer *et al*, 2005).

THERAPEUTIC USE

Amphetamine is a Schedule II drug, indicating that it has a high potential for abuse that can lead to dependence, but has wide medical use (DEA, 2002). While low doses (usually D and/or L amphetamine) are typically ingested orally for therapeutic purposes, high doses of amphetamine, especially methamphetamine, tend to be injected or smoked, and are associated with addiction and cognitive deficits (however, see Abuse-Academic Doping section of Methylphenidate for discussion of the illicit consumption of Adderall for academic doping).

Amphetamine is often prescribed for a number of diagnosable conditions, including narcolepsy, shift work sleep disorder, and, most commonly, ADHD. ADHD consists of a combination of behaviors that fall within the diagnostic criteria of inattention, hyperactivity, and impulsivity (American Psychiatric Association, 2000). A recent meta-analysis estimated the global prevalence of ADHD to be 5.29%, varying significantly by region (Polanczyk *et al*, 2007). By 2003, ADHD had been diagnosed in an estimated 11.0% of boys and 4.4% of girls 4-17 years old in the US (Visser and Lesesne, 2005). A more recent estimation set the number of ADHD diagnoses at 8.4% of all children between the ages of 6 and 17 years (Pastor and Reuben, 2008). Of those diagnosed, over half were currently taking medication for ADHD.

Stimulants are a first-line treatment for ADHD, with drugs such as Adderall and various preparations of methylphenidate (Concerta, Focalin) providing high levels of efficacy (Faraone and Biederman, 2002; Pietrzak *et al*, 2006). Stimulant medication

use in US youth has been increasing over the decades, with 0.6% of all surveyed youth using in 1987, jumping to 2.4% in 1996 (Olfson *et al*, 2002). In recent years, the prevalence of prescription stimulant use among children 18 years old and younger has been estimated from 2.2 million children, or 2.9% of the youth population (Zuvekas *et al*, 2006), to approximately 2.5 million children (Visser *et al*, 2005), demonstrating that legal stimulant use in the U.S. is pervasive.

In an early meta-analysis, Adderall, mixed D,L-amphetamine salts, reliably effected a large improvement in ADHD symptoms, compared to placebo. This improvement was consistent over different dosing regimens and scales of measurement (Faraone *et al*, 2002). In addition, a double-blind, placebo-controlled, crossover study examined the effects of Adderall (0.15 mg/kg and 0.3 mg/kg) in 154 children ranging in age from 5 to 16 years (Ahmann *et al*, 2001). Adderall was shown to have an efficacy rate of 59%, when examined with the criteria that parents and teachers agreed in their evaluation of the child's behavior. Adderall had an efficacy rate of 81%, when based on parental feedback, alone. Appetite-suppression, nausea, insomnia, and headaches were some of the side effects reported by parents of children taking Adderall, while higher levels of staring/daydreaming, and sadness/unhappiness were reported for children on placebo. A randomized, double-blind, crossover study of 35 children aged 6-12 years demonstrated a high level of efficacy for three types of amphetamine medications, including Adderall, compared to placebo (James *et al*, 2001). Another study found similarly effective results with extended release amphetamine (Adderall XR) in 258 adolescents with ADHD, ages 13 to 17 years

(Spencer *et al*, 2006). Participants were randomly assigned to 1 of 5 groups, 1 receiving placebo and 4 receiving Adderall XR (10 mg/d, 20 mg/d, 30 mg/d, or 40 mg/d), with doses in the higher dose groups being escalated throughout the 4-week experiment. All Adderall XR groups showed improvement in ADHD symptoms as assessed by both the ADHD Rating Scale-IV (ADHD-RS-IV) as well as the Clinical Global Impressions-Improvements (CGI-I) for ADHD, compared to placebo. Side effects, such as insomnia, headaches, abdominal pain, and weight loss had an increased prevalence in the Adderall XR groups, but were typically mild or moderate in their intensity.

Another school study compared the efficacy of daily Adderall XR (10 mg/d escalated to 30 mg) with that of Strattera (atomoxetine; 0.5 mg/kg escalated to 1.2 mg/kg), a popular "non-stimulant" treatment for ADHD, in 215 schoolchildren aged 6 to 12 years (Wigal *et al*, 2005). Over the course of the three weeks of the study, both medications led to improvement on a number of behavioral measures (e.g., academic productivity, attention), but Adderall led to greater gains in these measures than Strattera.

While the benefits of Adderall seem robust, a study found that ADHD patients on Adderall, atomoxetine, or Concerta (methylphenidate) did not perform neurocognitive tasks on par with control participants, despite performing better than untreated ADHD patients (Gualtieri and Johnson, 2008). In addition, there has been speculation regarding Adderall's potentially deleterious effects on creativity. This topic warrants further research, although one preliminary study found no evidence for

stunted creativity in those taking Adderall, with some data actually indicating an increase in creativity (Farah *et al*, 2009).

Off-label use of stimulants has also revealed therapeutic results. Ten participants with schizophrenia, currently taking antipsychotics, were administered 0.25 mg/kg D-amphetamine before a series of cognitive tasks. D-amphetamine improved reaction time on spatial working memory and Stroop tasks in both participants with schizophrenia and controls, and increased language production and improved working memory accuracy in those with schizophrenia (Barch and Carter, 2005). L-amphetamine, administered orally in increasing doses throughout a 29 d period (5 mg for days 1-7, 15 mg for days 8-14, and 30 mg for days 15-29), also enhanced verbal and spatial memory in cognitively impaired multiple sclerosis patients (Morrow *et al*, 2009). D-amphetamine, compared to placebo in a double-blind study, enhanced recovery from aphasia in stroke patients who were administered 10 mg of drug 30 min before speech therapy for 1 week (Walker-Batson *et al*, 2001).

ABUSE

Problems associated with chronic intake of high doses of amphetamine are well documented (e.g., Rogers and Robbins, 2001). A recent meta-analysis found that for cognitive deficits in participants with histories of long-term methamphetamine abuse or dependence, the largest effect sizes were in abilities related to learning and memory, as well as executive functioning (Scott *et al*, 2007). Converging evidence also points to deficits in social-cognitive functioning, which could compound the difficulties in daily living for those recovering from methamphetamine addiction

(Homer *et al*, 2008). For example, a group of adults with a history of methamphetamine dependence displayed significant impairment on social-cognitive tasks (facial affect recognition, theory of mind), after an average of 6 months of abstinence (Henry *et al*, 2009).

Converging evidence indicates that frontal brain areas mediate these cognitive deficits. One study demonstrated that methamphetamine dependent adults, abstinent for an average of 3 weeks, showed dysfunctional decision-making, relying more on an outcome-dependent (win-stay/lose-shift) strategy than controls on a two-choice prediction task (Paulus *et al*, 2002). The difference between groups decreased with longer periods of sobriety. Functional imaging showed that the methamphetamine-dependent participants had a pattern of hypofrontality, with diminished activation in a host of frontal regions during the task (inferior prefrontal, left prefrontal, bilateral ventromedial prefrontal, and right orbitofrontal cortex). Another study gathered resting PET scans on 24 abstinent methamphetamine-dependent males, finding significant hypometabolism in the left inferior frontal white matter, compared to 21 male controls (Kim *et al*, 2009). Using diffusion tensor imaging (DTI), methamphetamine users were found to display lower fractional anisotropy (FA), an indicator of white matter integrity, in frontal areas (Chung *et al*, 2007). For the male participants of this study, right frontal white matter correlated negatively with the number of errors on the Wisconsin Card Sorting Task, another test thought to measure frontal lobe function.

The corpus callosum has also been implicated in cognitive deficits in methamphetamine abusers. A structural MRI study found a number of differences within regions of the corpus callosum (e.g., increased curvature of the genu, decreased width of the posterior midbody and isthmus) of abstinent methamphetamine users compared to controls (Oh *et al*, 2005). A DTI study examining the corpus callosum in methamphetamine-dependent volunteers found that FA measures of the genu correlated with performance on the Stroop task in the methamphetamine users, but not in controls (Salo *et al*, 2009). While there was no significant group difference in the genu FA between users and nonusers ($p = .09$), these results indicate that the more deterioration in the genu of the corpus callosum, the worse the performance on cognitive control tasks, such as the Stroop interference task.

SUMMARY

> *Therapeutic doses are normally given up to about 60mg. It all depends on tolerance. Why not take some then take more till your f-ed up. You could grind it up into powder and snort or swallow that so the effects are quicker. [I] normally [take] 40-60mgs xr crushed up and snorted, when [I] want to get real f-ed its more like 100mg's (not recommended) but my tolerance is quite high. Just do like 40mg's then take some more till you feel good. remember to wait a bit till it hits you. ... [I have] never gone over 40mg, but based on the experiences of others who have, [I recommend] this estimated dosing schedule: (1) Light increase in motivation: 10mg-15mg. (2)'Good' club buzz: 20mg-40mg (add 1-2 drinks and [you are] set!). (3) Highway speeds: 60mg-80mg (might start cleaning the club/party your at, lol). (4) TWEAKED OUT: 100mg-120mg (not recommended). **Based on Instant-release pills take orally... as always tolerance and body-type depending…*[2]

[2]http://www.drugs-forum.com/forum/showthread.php?t=26171&highlight=recreational+amphetamine+dose (retrieved on 9/6/2010). Edited for spelling and grammar. It is common on these forums to use the acronyms "SWIM" (someone who isn't me) to designate yourself, and "SWIY" (someone who isn't you) when giving advice to others. For grammatical clarity, these have been edited to match the intent of the writer. See http://www.urbandictionary.com/define.php?term=SWIM (retrieved on 9/6/2010)

While much research has been devoted to studying the effects of low dose, prescription amphetamine, and separate research has investigated the effects of high dose, street amphetamine, little research has examined the effects of both low and high doses in the same study. To examine the boundary between the cognitive impairments and enhancements seen with amphetamine use, we examined the dose-response curve for D-amphetamine (0.005 – 8 mg/kg) on fear conditioning in rodents (Wood *et al*, 2009). In line with the effects of amphetamine seen in the literature discussed above, we found memory enhancements in mice administered low doses of amphetamine (0.005, 0.025, 0.05 mg/kg, i.p.), while memory impairments were evident in those administered moderate to high doses of amphetamine (8 mg/kg, i.p.; Figure 2). Interestingly, D-amphetamine only produced significantly locomotor hyperactivity at 4 and 8 mg/kg, well beyond the range where it produced memory enhancement (Figure 1). This further supports the idea that amphetamine's performance enhancing effects are dissociable from its effects on lcoomotor activity.

## METHYLPHENIDATE

### HISTORY OF USE

The Journal of the American Medical Association's Council on Drugs announced the introduction of methylphenidate (Ritalin) in its "New and Nonofficial Drugs" section in 1957 (Kautz, 1957). The report specified methylphenidate to be a

"central nervous system stimulant…less potent than amphetamine but more so than caffeine." The report also overly optimistically proclaimed that the effects of methylphenidate "on the gastrointestinal tract are negligible, and, unlike amphetamine, it does not produce anorexia." Subsequently, doctors used methylphenidate to combat a host of ailments. Intravenous methylphenidate (10 to 30 mg, three times daily) improved the majority of 164 patients manifesting a variety of symptoms including sleepiness, tremors, drooling, and nasal congestion (Ferguson *et al*, 1956). Methylphenidate (50 mg, i.v.) was also used to increase blood pressure in a comatose woman who had attempted suicide by overdose on the sedative hypnotics ethclorvynol (Placidyl) and methyprylon (Noludar), mixed with alcohol (Ivey, 1958). Startlingly, methylphenidate (0.4 mg/kg, i.m.) was injected into newborn infants with "depression," describing poor breathing, resulting in a "marked increase in respiratory activity" and "increased crying and bodily activity" (Gale, 1959).

Today, methylphenidate is most commonly prescribed for treatment of ADHD, and the number of prescriptions has been increasing over the decades. Between 1971 and 1987, in Baltimore county, methylphenidate rose from 40% to 93% of the total stimulants prescribed for ADHD (Safer and Krager, 1988). From 1990 to 1993, the number of outpatient visits for ADHD in the U.S. increased from 1.6 to 4.1 million, while the number of prescriptions for methylphenidate as a percent of total ADHD prescriptions increased from 67% to 71% (Swanson *et al*, 1995).

MECHANISM OF ACTION

Methylphenidate is a piperidine derivative whose structure and pharmacological properties are similar to those of amphetamine (Westfall *et al*, 2006). In vivo microdialysis studies in rats have helped clarify the mechanism of action of the drug. Methylphenidate (0.25, 0.5, and 1.0 mg/kg, i.p.) was found to dose-dependently increase extracellular levels of DA and NE in the prefrontal cortex (Berridge *et al*, 2006). The higher doses (0.5 and 1.0 mg/kg) led to an increase in DA in the nucleus accumbens, while the lowest dose (0.25 mg/kg) had no effect. Very high doses (10 and 20 mg/kg, i.p.) of methylphenidate have also been found to increase both NE in the prefrontal cortex and DA in the striatum (see Heal *et al*, 2009 for review of pharmacological profiles of popular ADHD medications). A range of doses of methylphenidate (1.0, 2.5, and 5.0 mg/kg, p.o.) increased norepinephrine in the hippocampus in a dose-dependent fashion, while only the highest dose, considered to exceed the therapeutic dosage, increased dopamine in the nucleus accumbens (Kuczenski *et al*, 2002). Another study determined the optimal dose of methylphenidate for each of 8 rats, as measured by improvement on the spatial delayed alternation task. For most rats, a lower dose (1.0 - 2.0 mg/kg, p.o.) improved performance, while higher doses (2.0 – 3.0 mg/kg, p.o.) often impaired performance (Arnsten and Dudley, 2005). The enhancing effects were reversed with co-administration of either the $\alpha 2$ antagonist, idazoxan, or the D1 antagonist, SCH23390. These findings suggest that methylphenidate improves performance by increasing the availability of NE and DA, which stimulate $\alpha 2$ and D1 receptors, respectively, in the

prefrontal cortex. Similar results were found for low dose methylphenidate locally administered in the lateral amygdala (Tye *et al*, 2010). Rats treated with methylphenidate, alone, displayed increased reward earning and task efficiency during an amygdala-dependent, cue-reward learning task. However, when SCH23390 was co-administered with methylphenidate, those enhancements vanished.

Human positron emission tomography (PET) studies agree with the animal literature in implicating dopamine as critical to the mechanism of action of methylphenidate. Oral methylphenidate was shown to block dopamine transporters in the human brain, with only approximately 0.25 mg/kg methylphenidate leading to 50% blockage of dopamine transporters (Volkow *et al*, 1998). Oral methylphenidate, still within the therapeutic range (0.8 mg/kg, on average), dramatically increased extracellular dopamine concentration, with the effect more pronounced in younger participants (Volkow *et al*, 2001).

THERAPEUTIC USE

Methylphenidate has been shown to be an effective treatment for ADHD. In a review of 40 articles on methylphenidate's effects on ADHD published since 1993, 63.5% of the studies identified improvements in cognitive function due to immediate-release methylphenidate (Pietrzak *et al*, 2006). Measures of planning/cognitive flexibility, attention/vigilance, saccadic eye movement, and inhibitory control showed improvement in the range of 70-83% of the studies. There is some evidence that these benefits may be seen exclusively when neural resources need to be recruited. For example, oral methylphenidate (40 mg) decreased the amount of glucose utilized to

perform a cognitive task, but did not affect glucose utilization under resting conditions that did not require cognitive effort (Volkow *et al*, 2008).

A pivotal study followed 103 children with ADHD over a two-year period, comparing three interventions: methylphenidate alone, methylphenidate plus multimodal psychosocial treatment, and methylphenidate plus attention control psychosocial treatment (Abikoff *et al*, 2004). Improvements in behavior were found across all groups, but, surprisingly, no additional benefit was found in those who had received psychosocial interventions in addition to drug treatment. These data helped to ensure that stimulants remain as the first line of intervention in treatment of ADHD.

A host of studies have found supporting results. Seventy-five children with ADHD, ages between 6-17 years, were administered between 5-20 mg/d D-methylphenidate (Focalin) during a 6-week, open label titration period, followed by a 2-week, double-blind placebo-controlled withdrawal period (Arnold *et al*, 2004). The primary measure of efficacy was the difference in CGI-I scores acquired during the last week of optimal dose administration compared to those gathered at the end of the withdrawal period. Participants administered placebo in the withdrawal period received ratings well below those of participants continuing with D-methylphenidate treatment. A similar pattern was found with behavioral ratings provided by teachers and parents, as well as with a math test. Another study of 132 children with ADHD, 6 to 17 years of age, found similar results when comparing the effects of D-methylphenidate (18.25 mg/d, average), D,L-threo-methylphenidate (32 mg/d, average), and placebo for 4 weeks (Wigal *et al*, 2004). Both teachers and parents

rated the participants' behavior as improved while on drug using the Swanson, Nolan and Pelham Rating Scale (SNAP). Generally, D-methylphenidate was found to be both safe and effective in the majority of participants.

As taking multiple doses of drug throughout the day can prove a hindrance to children in school, leading to less compliance, more efforts are being made to create extended release tablets. A study comparing extended-release D-methylphenidate (20 or 30 mg/d) and extended-release racemic methylphenidate hydrochloride (40 or 60 mg/d) with placebo in 84 children with ADHD, ages 6-12 years, also found significant improvement in attention and behavior after intake of either medication (Muniz *et al*, 2008). Measures of change from pre-dose rating on Swanson, Kotkin, Agler, M-Flynn, and Pelham (SKAMP) Rating Scale-Combined to a rating at different intervals post-dose demonstrated that extended-release dexmethylphenidate was faster acting at improving attention and behavior, while the extended-release racemic methylphenidate hydrochloride provided less dramatic, but longer-lasting improvement, seen at 10, 11, and 12 hours, post-dose. Similar findings were also reported for adolescents (n=177), ages 13 to 18 years, in a study of the efficacy and safety of osmotic-release oral system (OROS) methylphenidate (Concerta; Wilens *et al*, 2006). Adolescents completed a titration period, after which they received Concerta (18, 36, 54, or 72 mg/d) or placebo. ADHD symptoms improved more with drug treatment than placebo, as measured by the investigator, parents, and adolescents, using the ADHD Rating Scale (ADHD RS).

ABUSE – ACADEMIC DOPING

As is true with amphetamine, methylphenidate is a Schedule II drug, considered to be both medically useful as well as to have the potential for abuse and dependence (DEA, 2002). The 2007 National Survey on Drug Use and Health reported that, in the U.S., an estimated 6.9 million people had used psychotherapeutic drugs for nonmedical purposes within the previous month, 1.1 million of those having used stimulants (SAMHSA, 2008).

While those addicted to other stimulants may abuse methylphenidate, the more common, nonmedical use of the drug is what is currently described as academic doping. Generally speaking, academic doping is use of stimulants to better scholastic performance, by increasing focus or decreasing the need for sleep. Evidence for academic doping can be found in early literature discussing the introduction of amphetamine (Benzedrine) to the United States. In discussing what ailments could benefit from treatment by Benzedrine, one doctor included a section on "Application in Normal Individuals" (Nathanson, 1942):

> Various studies indicate that Benzedrine increases intelligence score under test conditions, and that psychomotor skill is increased. It is true that the improper use of the drug for this purpose has led to considerable publicity, and much warning as to possible harmful effects. The wide-spread and indiscriminate use by students in preparation for examinations is an illustration of improper usage.

Today's students who use prescription stimulants illicitly for studying seem to feel justified in doing so, separating themselves from those who use what are perceived as harder, or "bad" nonprescription drugs (DeSantis and Hane, 2010).

The prevalence of trivializing the use of prescription drugs illegally is evidenced in the data gathered by researchers over the past decade. A nationwide survey of 10,904 randomly selected students from 199 U.S. colleges and universities in 2001 revealed a lifetime prevalence of nonmedical stimulant use to be 6.9%, with 4.1% using within the previous month (McCabe *et al*, 2005). A survey of 3,401 first year students at a large, public university found that an estimated 13.3% had used prescription stimulants nonmedically at least once in their lives (Arria *et al*, 2008). Another survey at a large, public, southeastern university found that of the 1,811 student participants, 34% had used ADHD medications nonmedically (DeSantis *et al*, 2008). A sample of 390 college students found that 7.5% had used prescription stimulants for nonmedical purposes within the past 30 days (Weyandt *et al*, 2009). Questionnaires collected from 381 students at a midwestern university revealed that 13.7% of participants (17% of men, 11% of women) had taken stimulants for nonmedical purposes (Hall *et al*, 2005). Another study at a large, midwestern university surveyed 4,580 undergraduates, revealing that 8.3% had used illicit prescription stimulants in their lifetime, and 5.9% in the past year (Teter *et al*, 2006). Of the users, 75.8% reported using Adderall and 24.5% reported using methylphenidate. An informal survey administered by *Nature* found that roughly 20% of its 1,400 respondents had used drugs for nonmedical reasons, and 62% of those users had taken methylphenidate (Maher, 2008). In all, a recent review of the literature found a total of 21 studies on the illicit use of prescription stimulants, including 113,145 participants (Wilens *et al*, 2008). Rates of stimulant misuse within

the preceding year ranged from 5% to 9% in school-age children, and 5% to 35% in college-age adults.

While students may perceive stimulants to provide the boost needed for success, evidence for scholastic improvement is lacking. Illicit users of prescription stimulants have been found repeatedly to achieve lower GPAs than their nonusing counterparts (Arria *et al*, 2008; McCabe *et al*, 2005; Wilens *et al*, 2008).

Patients who are prescribed stimulants may abuse those stimulants, as well. A study surveying 545 patients in an ADHD treatment center revealed that 14.3% of respondents had abused prescription stimulants (Bright, 2008). Of those who had abused, 79.8% opted for short-acting agents, while 17.2% chose long-acting stimulants; 75% preferred crushing pills and snorting them over injection or other methods.

SUMMARY

> *I usually snort my Ritalin if I'm doing it for fun…I've found taking it before school to be very beneficial, and it actually makes what I'm learning almost seem interesting. It really does increase my attention span at lower doses. On the flip-side, higher doses (over 20 mg) usually send me into super-deep thought chains. If I'm very high on Ritalin, I'm usually too busy listening to my own thoughts race to listen to my teachers. It may help me pay attention and makes me more creative, but it won't get me into Harvard.*[3]
>
> *This morning, after I woke up, I took four 5 mg pills (20 mg Focalin) and it was fantastic. I felt like I was on [Adderall]. I felt highly euphoric, and found myself very easily absorbed in things…My hair felt like it was tingling, and I found myself completely enthralled with everything I was doing and it made my concentration level soar. I waited five hours and took another 15 mg. After about 20 minutes I started to feel really warm and my face became red, and my forehead was burning up. My body started to feel a bit numb, almost elevated,*

[3] http://www.erowid.org/experiences/exp.php?ID=15261 (retrieved on 9/6/2010)

> *and I started to feel out of body and started to panic a bit…I'm still feeling the effects as I type this…I find myself seeing small blips of light out of the corner of my eyes, and things shifting and I am lightly going in and out of consciousness.*[4]

Methylphenidate is commonly prescribed for a host of medical conditions, typically safely and effectively. Illicit use of this drug tends to involve academic doping, rather than self-administration of high doses and addiction, as seen with cocaine and amphetamine. However, self-administered at high doses, or using rapid routes of administration, methylphenidate can also lead to the subjective "high" and cognitive deficits found in the more addictive stimulants, such as cocaine or amphetamine.

## MODAFINIL

### HISTORY OF USE

Modafinil (Provigil, Modiodal, Nuvigil) is a psychostimulant that was developed to treat narcolepsy (Bastuji and Jouvet, 1988) and has emerged as the leading therapeutic used to treat the disorder. Modafinil is also approved for use with obstructive sleep apnea/hypopnea disorder and shift-work sleep disorder. Recently, numerous off-label applications have been tested including the treatment of ADHD, Alzheimer's disease, Parkinson's disease, depression, and stimulant addiction.

### MECHANISM OF ACTION

Modafinil was originally classified as a non-amphetamine psychostimulant because its pattern of activation was shown to be distinct from the more typical

[4] http://www.erowid.org/experiences/exp.php?ID=32144 (retrieved on 9/6/2010)

psychostimulants (Engber *et al*, 1998; Lin *et al*, 1996), though subsequent evidence has indicated that it may rely on similar mechanisms. Modafinil has actions on dopamine, norepinephrine, serotonin, glutamate, and GABA neurotransmission, however a clear mechanism has not emerged. The primary action of modafinil is generally considered to be on DA and/or NE signaling, with secondary changes in other systems (Minzenberg and Carter, 2008). It is unclear, however, whether catecholamine transporters or receptors mediate the primary effects of modafinil.

Evidence has been mixed since the early studies on modafinil as to whether catecholamine transporters or receptors underlie its action. An early study indicated that modafinil had only a weak affinity for the DA transporter, in comparison to reference compounds such as the DA reuptake blocker nomifensine (Mignot *et al*, 1994), indicating that this was unlikely to be the primary action of the drug. In contrast, DAT knockout mice failed to show the wake-promoting effects of modafinil (Wisor *et al*, 2001). These mice, however, also have reduced levels of D1 and D2 receptors, making it impossible to rule out the involvement of DA receptors in the study's results (Fauchey *et al*, 2000). Also, nomifensine did not alter modafinil-evoked currents in acutely isolated neurons, indicating the action of modafinil may be distinct from the DA transporter (Korotkova *et al*, 2007). Furthermore, the wake-promoting effects of modafinil are attenuated in D2 receptor knockout mice, and are completely abolished in these mice when combined with a D1 receptor antagonist (Qu *et al*, 2008). The authors interpret these findings as evidence that D1 and D2 receptors are essential for the arousal effect of modafinil, however this study is also consistent

with modafinil as a DA transporter blocker. Blocking the DA transporter would increase extracellular DA, but the DA would be unable to bind to D1 or D2 receptors. Thus, while D1 and D2 receptors appear to be involved in the actions of modafinil, the direct target remains unclear. A recent PET study, however, indicated that modafinil can bind to both DA and NE transporters at clinically relevant doses (2-8 mg/kg), and occupy the DA transporter to a comparable extent as methylphenidate (Madras *et al*, 2006). This finding indicates that DA and NE transporter inhibition remains a viable mechanism for the action of modafinil. Furthermore, a recent in vitro binding study indicated that modafinil selectively binds to DA transporters, with no affinity for DA receptors (Zolkowska *et al*, 2009). The authors also demonstrated that modafinil attenuated methamphetamine-induced locomotor activity and dopamine release. Finally, they established a strong correlation between modafinil-induced extracellular DA release and locomotor activity. Together, these findings indicate that modafinil acts as a weak inhibitor of the DA transporter. Thus, while modafinil may have some direct actions on dopamine receptors, current evidence suggests that the primary mechanism of action of modafinil is inhibition of dopamine transporters.

THERAPEUTIC USE

Modafinil is currently approved for the treatment of narcolepsy, obstructive sleep apnea/hypopnea disorder, and shift-work sleep disorder (Cephalon, 2004). Multiple randomized, double-blind, placebo controlled studies have confirmed the efficacy of modafinil in treating excessive daytime sleepiness (EDS) associated with narcolepsy (Bastuji *et al*, 1988; Billiard *et al*, 1994; Broughton *et al*, 1997; Fry *et al*,

1998; Gross *et al*, 2000), ensuring its emergence as the leading pharmacological therapeutic. Clinical trials have also shown modafinil to be effective in the treatment of obstructive sleep apnea/hypopnea disorder (Black and Hirshkowitz, 2005; Kingshott *et al*, 2001; Pack *et al*, 2001) and shift-work sleep disorder (Czeisler *et al*, 2005; Erman *et al*, 2007).

The unknown mechanism of action and minimal side effect profile has made modafinil a prime candidate for a variety of investigational uses. Moreover, modafinil is a schedule IV drug in the U.S., thought to reflect low abuse potential, making it easier to prescribe. Medical uses have been reviewed recently (Kumar, 2008) and include treating ADHD, depression, bipolar disorder, schizophrenia, cocaine addiction, general fatigue, as well as EDS in Parkinson's disease, myotonic dystrophy, and traumatic brain injury, Many clinical trials were completed to test the efficacy of modafinil in treating these disorders, however many of them suffer from inconsistent findings and small sample size. The most consistent positive results for modafinil were in the treatment of ADHD in children and adolescents. Three large, double-blind, placebo controlled clinical trials concluded that modafinil (170-425 mg/day) was an effective treatment, with primary and secondary efficacy measures of ADHD significantly decreasing more than placebo controls (Biederman *et al*, 2005; Greenhill *et al*, 2006; Swanson *et al*, 2006). A separate study compared modafinil (200-300 mg/day) to methylphenidate (20-30 mg/day) and found that both drugs effectively reduced symptoms, and no differences were found between the two drug groups (Amiri *et al*, 2008). Thus, modafinil appears to be effective at treating ADHD in

children and adolescents, and the most serious side of effects of methylphenidate and amphetamine (on blood pressure and appetite) are greatly reduced. However, due to very rare suspected cases of Stevens-Johnson syndrome during the ADHD clinical trials, the FDA failed to approve modafinil, to be marketed under the trade name Sparlon, for this indication (Cephalon, 2006; FDA, 2007). Modafinil was also effective at treating EDS in myotonic dystrophy (MacDonald *et al*, 2002; Talbot *et al*, 2003; Wintzen *et al*, 2007). All other therapeutic applications that were examined produced inconsistent findings or were inconclusive because of extremely small sample sizes (Kumar, 2008).

ABUSE

Modafinil is an attractive therapeutic because it appears to have limited abuse potential (Myrick *et al*, 2004). There are no reported cases of addiction to modafinil and several reports have indicated that at therapeutic doses, the drug dose not produce euphoria (Malcolm *et al*, 2002; Rush *et al*, 2002). Several factors may contribute to this lack of euphoria including a relatively slow onset and a long half-life (10-12 hours), compared to stimulants of abuse. It remains possible, however, that high doses of modafinil, especially if given via a rapid route of administration, could be addictive. Indeed, high doses of modafinil (7-10 mg/kg) have been reported to increase "liking" and experiences of a "high" equivalent to methylphenidate (Jasinski, 2000) and D-amphetamine (Makris *et al*, 2007). In rodents, initial studies indicated that modafinil was not reinforcing when administered alone (Deroche-Gamonet *et al*, 2002), however we have recently found that high dose modafinil (75 mg/kg, i.p.) alone can produce a

conditioned place preference (Shuman *et al*, submitted), indicating that modafinil is at least a weak reinforcer. Consistent with this profile, modafinil modestly increases extracellular dopamine in the nucleus accumbens in both rats and humans (Ferraro *et al*, 1997; Ferraro *et al*, 1996; Volkow *et al*, 2009).

A common abuse of modafinil is academic doping (Garreau, 2006), similar to amphetamine and methylphenidate (see further discussion in Methylphenidate – Abuse – Academic Doping section). A number of studies have reported increased cognition and attention in humans (Muller *et al*, 2004; Turner *et al*, 2003) and rodents (Beracochea *et al*, 2001; Beracochea *et al*, 2002; Beracochea *et al*, 2003; Morgan *et al*, 2007; Shuman *et al*, 2009; Ward *et al*, 2004; Waters *et al*, 2005).

SUMMARY

> *"For me, I started with 100 mg and am still at that level after 2 months…Sleep will probably be a problem the first couple of days, even if you only take a dose in the morning, but you'll adjust in a few days. You will probably end up cleaning your whole house those first few days, waxing and detailing your car, etc. Enjoy! I no longer experience that physical energy lift, but mental and emotionally I still get great benefit from Provigil. Try doing some Sudoku puzzles or crossword puzzles and see if you find them easy and enjoyable while on Provigil – I know I do."*[5]

A sharp discord exists between the doses of modafinil studied in humans and in rodents. Human studies have focused on clinically relevant doses (100 – 400 mg = 1.25 – 5 mg/kg) while rodent studies have used a very large range of doses, focusing on the highest doses (generally 32 – 128 mg/kg). Indeed, some effects of modafinil do not appear until these high doses, other effects may be overlooked. We recently completed a dose-effect analysis of modafinil and its memory enhancing effects using

[5] http://www.dr-bob.org/babble/20080412/msgs/823572.html (retrieved on 7/27/10)

multiple doses ranging from below the clinically relevant dose (0.075 mg/kg) to the highest dose we could give without noticeable side effects (75 mg/kg). We found that the dose closest to the clinically prescribed dose (0.75 mg/kg) was able to enhance memory, while the highest dose (75 mg/kg) disrupted the memory (Figure 2; Shuman *et al*, 2009). Thus, there were clear dose-dependent effects of modafinil. In addition, the lowest dose of modafinil (0.075 mg/kg) was able to significantly reduce locmotor activity, despite being $1/1000^{th}$ of the dose that is typically tested in rodents (Figure 1). In our hands, even the highest dose of modafinil (75 mg/kg) failed to produce locomotor activity, but modafinil can also produce some hyperactivity at high doses, particularly when the subjects have been habituated to the training context (Simon *et al*, 1996; Simon *et al*, 1994; van Vliet *et al*, 2006; Zolkowska *et al*, 2009).

## CAFFEINE

### HISTORY OF USE

Caffeine is found naturally in over 60 plants, with the most popular utilized in the production of coffee, tea and cocoa. While the details surrounding the discovery of coffee are still debated, it is certain that coffee consumption was present thousands of years ago in both Africa and the Arabian Peninsula (see Smith *et al*, 2007 for a detailed history of coffee, as well as other caffeinated consumables such as tea, chocolate, and soft drinks).

### MECHANISM OF ACTION

Caffeine is a legal stimulant used widely around the world, typically not considered a drug of abuse (Graham, 2001). Unlike many of the other stimulants

discussed above, caffeine does not have its primary actions on the dopamine receptor, but on subtypes of the adenosine receptor. Specifically, caffeine is a nonselective antagonist, acting on the $A_1$ and $A_{2A}$ receptor subtypes (Takahashi *et al*, 2008).

USE TODAY

Caffeine is typically consumed in drinks such as coffee, tea, and soda, although today its presence is ubiquitous, with caffeine found in products such as breath mints, lip balm, and shampoo (Bramstedt, 2007). The per capita daily intake of caffeine in the U.S. population has been estimated to be 3 mg/kg, or roughly 200 mg of caffeine for a 65 kg person, with the heaviest consumers ranging from 5 to 7 mg/kg, or approximately 325 to 450 mg caffeine (Barone and Roberts, 1996). It also has been estimated that a 5 oz (~150 ml) cup of coffee contains between 60 and 85 mg of caffeine, although the same amount of coffee has been reported to contain anywhere between 21 to 176 mg, depending on the preparation of the beans and the type of drink (Barone *et al*, 1996). It is worth noting that a "small" coffee sold today in the U.S. is typically between 8 – 12 oz (230 – 350 ml), indicating that a single serving of caffeine might be more accurately estimated at twice the previously reported amounts (roughly 120 to 170 mg).

With such heavy consumption throughout society, it is relevant to determine the health effects of caffeine at regularly consumed doses. Unlike most of the other stimulants discussed herein, a review of the literature on caffeine found its habitual consumption to be quite safe, revealing no adverse on a number of health measures, including cardiovascular health, increased incidence of cancer, and calcium balance

(Nawrot *et al*, 2003). Although the literature is mixed, women who are pregnant or attempting to become pregnant, as well as children, seem to be populations at higher risk, with female fertility and fetal growth possibly adversely affected by moderate caffeine consumption at doses up to 400 mg per day, or roughly 6 mg/kg in a 65 kg person. The authors also discovered that caffeine is unlikely to have teratogenic effects in the human fetus, although some animal literature is apparently in contradiction to these findings, demonstrating fetal malformations after caffeine intake.

The incongruous results found in the realms of animal and human research is a common problem throughout the research performed on stimulants, in general, and is worth discussing here. In this example, a review article noted that animal studies have shown caffeine to have teratogenic effects at doses ≥ 80 mg/kg (Nawrot *et al*, 2003). In humans, one gram (roughly 15 mg/kg in a 65 kg person) of caffeine is able to induce hallucinations, while five grams (roughly 75 mg/kg) can be fatal (Bramstedt, 2007). With this in mind, it should come as no surprise that a dose of caffeine that is potentially lethal in humans produces teratogenic effects in the rat fetus. In comparing human and animal research on drugs, it is important to keep in mind the different doses being administered, and the possible effects on the results and conclusions drawn from the studies. A similar argument can be made in regards to the route of administration of drugs in animal (typically i.p. injection) and human (typically oral) pharmacology research (see Kuczenski and Segal, 2005 for an in-depth discussion of these issues as they relate to ADHD pharmacotherapy research).

Along the same lines, another issue worth noting in the caffeine literature is the variability in methods used to determine dose. In many studies, a single amount of caffeine is administered to all participants, regardless of weight. As Graham pointed out, this could lead to females in a study receiving a dose of roughly 20% higher than men, due to their overall smaller bodyweight (2001). It is less common for doses to be administered to humans in mg/kg doses, while that is the norm in animals.

Finally, another common problem that may selectively taint caffeine data is that of withdrawal effects. The "withdrawal reversal hypothesis" (Rogers and Dernoncourt, 1998) states that caffeine does not enhance cognition or attention, but it reverses the negative effects of caffeine withdrawal in those who typically consume caffeine daily. Many studies ask their participants to not ingest any caffeine for a set time before the study, typically around 24 hours, leaving the participants in a withdrawal state if they habitually ingest caffeine. Evidence for this hypothesis can be found scattered throughout the literature. A 200 mg dose of caffeine was found to improve performance on a difficult multiplication task compared to 400 mg or placebo, although habitual caffeine use (low, moderate, or high; less then about 55mg/day, between approximately 56 and 132 mg/day, or above roughly 133 mg/day, respectively) was a more important factor on word recall, with high to moderate caffeine users remembering more words (Loke, 1988). An interaction was found in typical level of caffeine consumption and performance on the RVIP task after caffeine consumption (Smit and Rogers, 2000). Participants who were lower consumers of caffeine (< 100 mg/day) did not show any benefit from consuming any dose of

caffeine (12.5, 25, 50 or 100 mg), compared to the higher consumers (> 200 mg/day), who uniformly demonstrated enhanced performance compared to controls after administration of any dose tested. In another study by the same group, participants were divided in two groups, one group of those who consumed virtually no caffeine, the "non-consumers", and the other group of those whose daily intake of caffeine averaged more than 200 mg, the "consumers" (Rogers *et al*, 2003). After administration of 100 mg caffeine or placebo, caffeine improved the performance of the simple reaction time (SRT) task in consumers, but not in non-consumers. The authors also pointed out that the three non-consumers whose SRT performance declined substantially after caffeine administration also reported large increases in jitteriness and tension. This study demonstrates that even a small to moderate amount of caffeine is able to affect fine motor tasks in those who do not typically consume caffeine.

Despite these potential confounds, much research has been conducted using different doses of caffeine on a variety of cognitive tasks. For example, 250 mg (~4 mg/kg) caffeine improved performance on the digit symbol substitution task (DSST), a test of perceptual speed and memory, more than a 500 mg (~8 mg/kg) dose, over placebo (Kaplan *et al*, 1997). A different study found that the relatively low doses of 12.5, 50 or 100 mg caffeine all enhanced performance of a simple reaction time (SRT) task, compared to controls (Smit *et al*, 2000). A low dose of caffeine (150 mg) was also found to improve the speed of digit vigilance reaction time, as well as the accuracy of Rapid Visual Information Processing (RVIP), a task extensively utilizing

working memory (Haskell *et al*, 2008). This study was the only one reviewed, herein, that took saliva samples from its participants to confirm abstinence from caffeine preceding the test days; however, no records were taken on habitual caffeine use.

Caffeine has also been found to affect declarative memory, with more varied results. For example, 2 mg/kg and 4 mg/kg caffeine impaired recall of a word list read one word every three seconds, but not one word every second, compared to placebo in female, but not male, participants (Erikson *et al*, 1985). In a study designed to replicate and expand upon these results, the opposite effect was found in females, with 2 and 4 mg/kg caffeine enhancing word list recall after practice (Arnold *et al*, 1987). In the same study, caffeine impaired word recall for males at 2 mg/kg at certain amounts of practice, while 4 mg/kg had no effect. Disruption of free-recall of word lists (Auditory Verbal Learning Task, AVLT) has also been reported after a 100 mg dose of caffeine, although participants were allowed to have any caffeinated beverage just three hours before testing, resulting in an unknown amount of caffeine actually consumed and processed during testing (Terry and Phifer, 1986). No difference was found between placebo and 200 mg caffeine for word recognition and recall after a 7-hour delay period (Mednick *et al*, 2008).

Animal studies are able to avoid a number of potential confounds commonly found in the human literature. Several studies have used the passive avoidance task in rodents to examine the effects of caffeine and adenosine receptor agonists and antagonists on learning. For example, the $A_1$ adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) infused (1, 25, or 50 nM) directly into the

posterior cingulate cortex in rats, post-training, significantly enhanced both short- and long-term passive avoidance retention at the 50 nM concentration (Pereira *et al*, 2002). However, when administered, i.p. (0.1, 0.3, 1.0, and 3.0 mg/kg) in mice, post-training, DPCPX had no effect on learning at any dose (Kopf *et al*, 1999). The discrepancy in results may lie in the different routes of administration used, with the direct infusion of DPCPX allowing the drug to bind more selectively than an i.p. injection.

When caffeine was used on the passive avoidance task (0.1, 0.3, 1.0, and 3.0 mg/kg, i.p.) in mice, the 0.3 mg/kg dose administered immediately but not 180 minutes following training produced a better performance on the test 24 hours later (Kopf *et al*, 1999). Interestingly, another study in mice found that doses of 10, 30 and 100 mg/kg administered 30 minutes before training impaired learning, while doses of 1, 3, 10, and 30 mg/kg, i.p., administered immediately following training enhanced learning (Angelucci *et al*, 1999). It is worth noting that the study by Kopf, et al. found no increase in learning with a 3.0 mg/kg dose of caffeine administered immediately after training, while Angelucci et al. (who used weaker training) did find a significant enhancement in learning with this dose. These findings demonstrate that while rodent studies are able avoid confounds such as caffeine pre-exposure, care still needs to be taken when comparing results.

The $A_1$ adenosine receptor agonist N6-cyclopentyladenosine (CPA) was effective at disrupting memory for Pavlovian fear conditioning (Corodimas and Tomita, 2001). Rats administered 1.0 or 1.5 mg/kg CPA, i.p., 30 minutes before fear conditioning training showed significant impairment in fear memory when tested for

contextual fear 24 hours after training. Fear to the tone was intact, however, indicating selective disruption of the acquisition of hippocampus-dependent learning. Caffeine (20 and 30, but not 10 mg/kg, i.p.) administered 15 minutes before training also disrupted contextual fear conditioning, with no significant effect on tone conditioning (Corodimas *et al*, 2000). Interestingly, no deleterious effects on contextual or cued learning were found with chronic administration of caffeine (5, 10, or 25 mg pellets of caffeine, s.c.) over the course of seven days. The authors hypothesize this could be due to a change in the number of adenosine receptors in areas such as the hippocampus and lateral nucleus of the amygdala, two areas critical for the acquisition and performance of fear conditioning.

POTENTIAL THERAPEUTIC USE

The greatest benefits of caffeine on cognition may lie in the realm of disease, with caffeine lending neuroprotective support against a host of conditions, ranging from the general effects of aging (Hameleers *et al*, 2000) to ADHD (Prediger *et al*, 2005). One community-based, observational study of older (50 years in age or older) adults found that lifetime coffee consumption in women was positively correlated with performance on measures of long-term memory, short-term memory, verbal fluency, and attention (Johnson-Kozlow *et al*, 2002). A study conducted in the Netherlands with a large number of participants (1,875) stratified for age (24-81 years) found a positive correlation between habitual caffeine consumption and measures of simple response speed and verbal long-term memory (Hameleers *et al*, 2000). The study, however, did not find an association between caffeine intake and short-term memory,

planning capacity, information processing, or attention. Data from a six-year follow up with the same cohort revealed that caffeine intake was not predictive of enhanced performance on the verbal long-term memory task, and that the effects on improvement of the motor task were small (van Boxtel *et al*, 2003).

Epidemiological evidence also indicates that caffeine consumption may be linked to a lower chance of developing Parkinson's disease (PD) in older women who never used postmenopausal hormones and in older men (Ascherio and Chen, 2003). Neurophysiological and behavioral research supports the validity of this trend, with $A_{2A}$ adenosine receptor antagonists implicated in the prevention of excitotoxicity in models of stroke and Huntington's disease through the suppression of excessive glutamate release throughout the cortex (Schwarzschild *et al*, 2003). In addition, $A_{2A}$ adenosine receptors densely populate the striatum. Converging evidence suggests blockade of these receptors may help protect the dopaminergic nigrostriatal neurons, whose destruction is the main cause of symptoms of PD (Schwarzschild *et al*, 2003).

Long-term caffeine consumption was also shown to have neuroprotective effects in a mouse model of Alzheimer's disease, the amyloid precursor protein Swedish mutation transgenic mice (Arendash *et al*, 2006). Caffeine was administered in the drinking water of the mice starting from four months of age throughout behavioral testing until sacrifice at 9 ½ months of age, at a rate of roughly 1.5 mg consumed daily (estimated by the authors to equate to 500 mg intake by humans, roughly 7 mg/kg, or five cups of coffee). Caffeine consumption provided cognitive protection in the Morris water maze, the platform recognition task, a hippocampus-

dependent reference memory task (circular platform task), and a working memory task (radial arm water maze). Moreover, caffeine seemed to reduce the production of hippocampal β-amyloid, a protein that is found in higher levels in those with AD. While the density of $A_1$ or $A_{2A}$ receptors throughout the cortex or hippocampus were not altered by caffeine, adenosine levels in the transgenic mouse brain were restored to those found in the wild type mouse brain. Caffeine (3 mg/day) also exhibited protective effects against the disruption of the blood brain barrier in a rabbit model of Alzheimer's disease (Chen *et al*, 2008). These effects are likely not mediated by an increase in neuron production, as a recent study found effects of caffeine on cell proliferation in the dentate gyrus, at very high doses, but no effects on survival or differentiation at any dose (Wentz and Magavi, 2009).

SUMMARY

Some potential confounds discussed above are seen throughout the stimulant literature (e.g., use of very high doses, with rapid routes of administration in animal studies with comparatively low doses and slow routes of administration in human studies) while others are specific to the caffeine literature (e.g., the "withdrawal reversal hypothesis"). Despite these issues, a general pattern emerges from the human and animal studies on caffeine. As found with the other stimulants reviewed herein, dose seems to be the primary determinant of caffeine's effects. In general, lower doses of caffeine lead to positive effects, while higher doses produce disruptive effects.

## A CONTINUUM OF PSYCHOSTIMULANT ACTIVATION

In summarizing these studies we have emphasized the role of dose in determining psychostimulant action. In borrowing from the conception of describing the action of sedative-hypnotics (e.g., Meyer *et al.*, 2005), we propose that psychostimulant action is best considered on a continuum (Figure 3). At low doses, stimulants produce an increase in wakefulness, attention, and an increase in confidence and vigor. Drugs with low potency or maximum effect, such as caffeine or modafinil, act much like low doses of amphetamine or methylphenidate. As dose or potency increase, hyperlocomotion is seen, with an increased sense of power, perhaps accompanied by mania. This is closely followed by euphoria, or the drug-induced high. This is the domain of the addict, who is likely to use high potency drugs such as cocaine or methamphetamine, and administer them rapidly to achieve this effect. These effects are well outside of the range of cognitive enhancement; in fact, deficits in cognition and disturbed thinking are usually observed. As overdose begins, agitation, confusion and psychosis are seen. At the very high doses, stimulants produce typical toxic effects, including coma, circulatory collapse, and, ultimately, death. Therefore, we here draw critical attention to dose, rather than particular drug, in terms of determining psychiatric efficacy of stimulants versus their liability for abuse and addiction. Low doses of even the most potent stimulants (e.g., methamphetamine) have been used for over fifty years with much success, but at the same time, reckless use of these drugs at high doses continues to be a social epidemic.

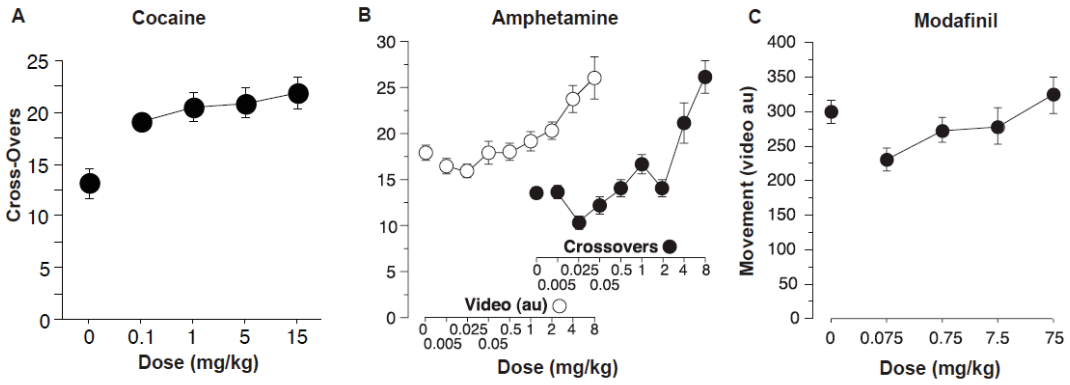


**Figure 1**

*Training baseline activity dose-response curves.* Activity was measured before training during a 2 min baseline period, while subjects were on drug. Dose-dependent increases in activity were seen in amphetamine and cocaine, but not modafinil. Figures adapted from Wood *et al.*, 2009, Wood *et al.*, 2007, and Shuman *et al.*, 2009.

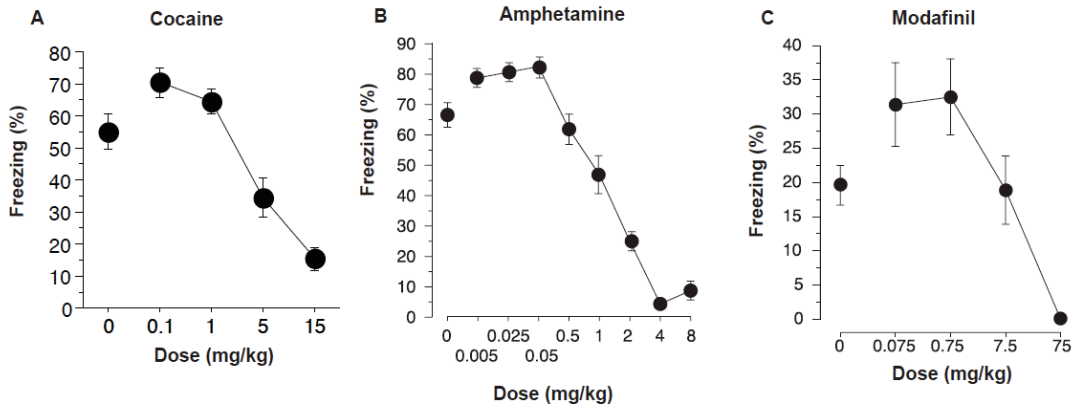


**Figure 2**

*Post-shock freezing dose-response curves.* Freezing was measured immediately following training, with dose-dependent effects on freezing seen with all drugs. All animals were on drug for this measure. Those administered low dose stimulants exhibited greater levels of freezing than their respective placebo controls. Figures adapted from Wood *et al.*, 2009, Wood *et al.*, 2007, and Shuman *et al.*, 2009.

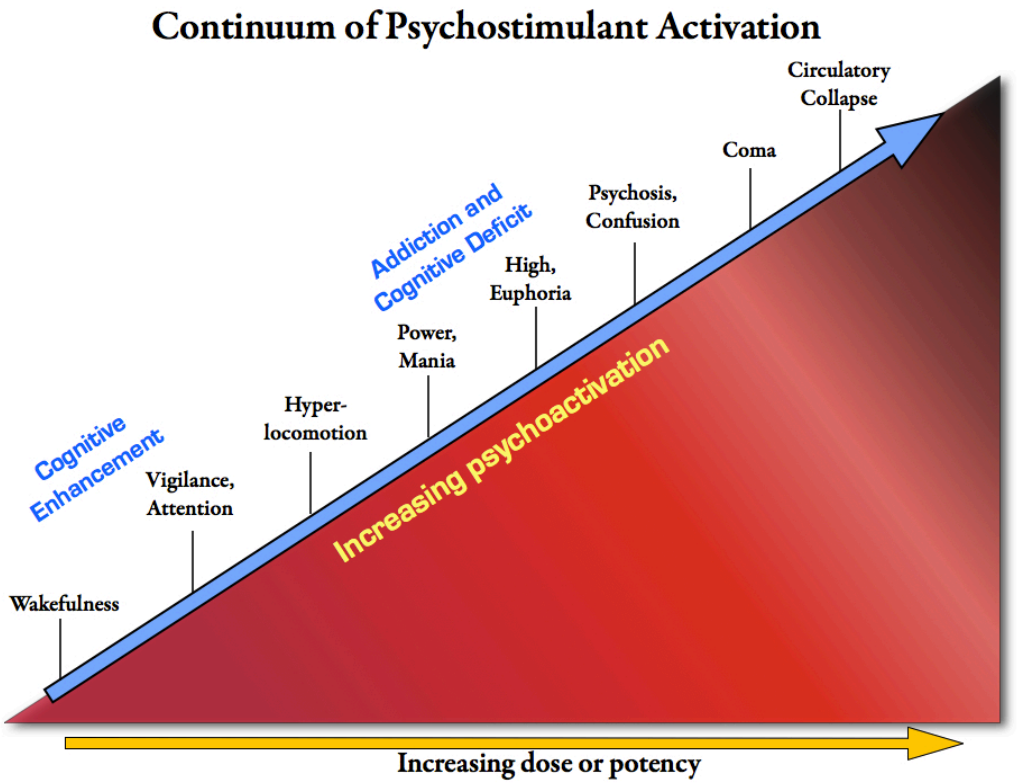


**Figure 3**
*Continuum of psychostimulant activation*. We propose a guideline for the effects of different doses of stimulants, beginning with increased wakefulness seen with low doses, and ending with addiction and death accompanying high doses.

REFERENCES

Abikoff H, Hechtman L, Klein RG, Weiss G, Fleiss K, Etcovitch J*, et al* (2004). Symptomatic improvement in children with ADHD treated with long-term methylphenidate and multimodal psychosocial treatment. *J Am Acad Child Adolesc Psychiatry* **43**(7): 802-811.

Abourashed EA, El-Alfy AT, Khan IA, Walker L (2003). Ephedra in perspective--a current review. *Phytother Res* **17**(7): 703-712.

Ahmann PA, Theye FW, Berg R, Linquist AJ, Van Erem AJ, Campbell LR (2001). Placebo-controlled evaluation of amphetamine mixture-dextroamphetamine salts and amphetamine salts (Adderall): efficacy rate and side effects. *Pediatrics* **107**(1): E10.

Al-Hebshi NN, Skaug N (2005). Khat (Catha edulis)-an updated review. *Addict Biol* **10**(4): 299-307.

Al-Motarreb A, Baker K, Broadley KJ (2002). Khat: pharmacological and medical aspects and its social use in Yemen. *Phytother Res* **16**(5): 403-413.

American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* American Psychiatric Press: Washington, D. C.

Amiri S, Mohammadi MR, Mohammadi M, Nouroozinejad GH, Kahbazi M, Akhondzadeh S (2008). Modafinil as a treatment for Attention-Deficit/Hyperactivity Disorder in children and adolescents: a double blind, randomized clinical trial. *Prog Neuropsychopharmacol Biol Psychiatry* **32**(1): 145-149.

Angelucci ME, Vital MA, Cesario C, Zadusky CR, Rosalen PL, Da Cunha C (1999). The effect of caffeine in animal models of learning and memory. *Eur J Pharmacol* **373**(2-3): 135-140.

Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, Cracchiolo JR*, et al* (2006). Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. *Neuroscience* **142**(4): 941-952.

Arnold LE, Lindsay RL, Conners CK, Wigal SB, Levine AJ, Johnson DE*, et al* (2004). A double-blind, placebo-controlled withdrawal trial of dexmethylphenidate hydrochloride in children with attention deficit hyperactivity disorder. *J Child Adolesc Psychopharmacol* **14**(4): 542-554.

Arnold ME, Petros TV, Beckwith BE, Coons G, Gorman N (1987). The effects of caffeine, impulsivity, and sex on memory for word lists. *Physiol Behav* **41**(1): 25-30.

Arnsten AF (2006). Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology* **31**(11): 2376-2383.

Arnsten AF, Dudley AG (2005). Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct* **1**(1): 2.

Arria AM, Caldeira KM, O'Grady KE, Vincent KB, Johnson EP, Wish ED (2008). Nonmedical use of prescription stimulants among college students: associations with attention-deficit-hyperactivity disorder and polydrug use. *Pharmacotherapy* **28**(2): 156-169.

Ascherio A, Chen H (2003). Caffeinated clues from epidemiology of Parkinson's disease. *Neurology* **61**(11 Suppl 6): S51-54.

Barch DM, Carter CS (2005). Amphetamine improves cognitive function in medicated individuals with schizophrenia and in healthy volunteers. *Schizophr Res* **77**(1): 43-58.

Barone JJ, Roberts HR (1996). Caffeine consumption. *Food Chem Toxicol* **34**(1): 119-129.

Bastuji H, Jouvet M (1988). Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. *Prog Neuropsychopharmacol Biol Psychiatry* **12**(5): 695-700.

Beracochea D, Cagnard B, Celerier A, le Merrer J, Peres M, Pierard C (2001). First evidence of a delay-dependent working memory-enhancing effect of modafinil in mice. *Neuroreport* **12**(2): 375-378.

Beracochea D, Celerier A, Borde N, Valleau M, Peres M, Pierard C (2002). Improvement of learning processes following chronic systemic administration of modafinil in mice. *Pharmacol Biochem Behav* **73**(3): 723-728.

Beracochea D, Celerier A, Peres M, Pierard C (2003). Enhancement of learning processes following an acute modafinil injection in mice. *Pharmacol Biochem Behav* **76**(3-4): 473-479.

Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B*, et al* (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol Psychiatry* **60**(10): 1111-1120.

Bickel WK, Miller ML, Yi R, Kowal BP, Lindquist DM, Pitcock JA (2007). Behavioral and neuroeconomics of drug addiction: competing neural systems and temporal discounting processes. *Drug Alcohol Depend* **90 Suppl 1**: S85-91.

Biederman J, Swanson JM, Wigal SB, Kratochvil CJ, Boellner SW, Earl CQ*, et al* (2005). Efficacy and safety of modafinil film-coated tablets in children and adolescents with attention-deficit/hyperactivity disorder: results of a randomized, double-blind, placebo-controlled, flexible-dose study. *Pediatrics* **116**(6): e777-784.

Billiard M, Besset A, Montplaisir J, Laffont F, Goldenberg F, Weill JS*, et al* (1994). Modafinil: a double-blind multicentric study. *Sleep* **17**(8 Suppl): S107-112.

Billman GE (1990). Mechanisms responsible for the cardiotoxic effects of cocaine. *FASEB J* **4**(8): 2469-2475.

Black JE, Hirshkowitz M (2005). Modafinil for treatment of residual excessive sleepiness in nasal continuous positive airway pressure-treated obstructive sleep apnea/hypopnea syndrome. *Sleep* **28**(4): 464-471.

Bolla K, Ernst M, Kiehl K, Mouratidis M, Eldreth D, Contoreggi C*, et al* (2004). Prefrontal cortical dysfunction in abstinent cocaine abusers. *J Neuropsychiatry Clin Neurosci* **16**(4): 456-464.

Bolla KI, Eldreth DA, London ED, Kiehl KA, Mouratidis M, Contoreggi C*, et al* (2003). Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. *Neuroimage* **19**(3): 1085-1094.

Bradley C (1937). The Behavior of Children Receiving Benzedrine. *Am J Psychiatry*(94): 577-585.

Bramstedt KA (2007). Caffeine Use by Children: The Quest for Enhancement. *Substance Use and Misuse* **42**(8): 1237-1251.

Bright GM (2008). Abuse of medications employed for the treatment of ADHD: results from a large-scale community survey. *Medscape J Med* **10**(5): 111.

Broughton RJ, Fleming JA, George CF, Hill JD, Kryger MH, Moldofsky H*, et al* (1997). Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. *Neurology* **49**(2): 444-451.

Cartmell LW, Aufderheide AC, Springfield AC, Weems C, Arriaza B (1991). The Frequency and Antiquity of Prehistoric Coca-Leaf-Chewing Practices in Northern

Chile: Radioimmunoassay of a Cocaine Metabolite in Human-Mummy Hair. *Latin American Antiquity* **2**(3): 260-268.

Catterall WA, Mackie K (2006). Chapter 14. Local Anesthetics. In: L. BL, Lazo JS, Parker KL (eds). *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition*. The McGraw-Hill Companies: New York.

Cephalon (2004). FDA Approved Labeling Text for NDA 20-717/S-005 & S-008. Provigil (modafinil) Tablets [C-IV], Approved 23-Jan-2004. In: F.D.A. (ed).

Cephalon (2006). Modafinil (CEP-1538) Tablets. Supplemental NDA 20-717/S-019. ADHD Indication. *Psychopharmacologic Drugs Advisory Committee Meeting*. Cephalon: Frazer, PA.

Chen X, Gawryluk JW, Wagener JF, Ghribi O, Geiger JD (2008). Caffeine blocks disruption of blood brain barrier in a rabbit model of Alzheimer's disease. *J Neuroinflammation* **5**: 12.

Chung A, Lyoo IK, Kim SJ, Hwang J, Bae SC, Sung YH*, et al* (2007). Decreased frontal white-matter integrity in abstinent methamphetamine abusers. *Int J Neuropsychopharmacol* **10**(6): 765-775.

Colzato LS, van den Wildenberg WP, Hommel B (2008). Reduced spontaneous eye blink rates in recreational cocaine users: evidence for dopaminergic hypoactivity. *PLoS One* **3**(10): e3461.

Corodimas KP, Pruitt JC, Stieg JM (2000). Acute exposure to caffeine selectively disrupts context conditioning in rats. *Psychopharmacology (Berl)* **152**(4): 376-382.

Corodimas KP, Tomita H (2001). Adenosine A1 receptor activation selectively impairs the acquisition of contextual fear conditioning in rats. *Behav Neurosci* **115**(6): 1283-1290.

Czeisler CA, Walsh JK, Roth T, Hughes RJ, Wright KP, Kingsbury L*, et al* (2005). Modafinil for excessive sleepiness associated with shift-work sleep disorder. *N Engl J Med* **353**(5): 476-486.

Davies IJ, Guttmann E, Trevan JW, Richter D, Rudolf GM, Strom-Olsen R (1939). Benzedrine: Uses and Abuses: (Section of Medicine with the Section of Therapeutics and Pharmacology). *Proc R Soc Med* **32**(4): 385-398.

DEA (2002). 21 USC § 812. In: Act CS (ed). *Drug Enforcement Administration*.

Deroche-Gamonet V, Darnaudery M, Bruins-Slot L, Piat F, Le Moal M, Piazza PV (2002). Study of the addictive potential of modafinil in naive and cocaine-experienced rats. *Psychopharmacology (Berl)* **161**(4): 387-395.

DeSantis AD, Hane AC (2010). "Adderall is definitely not a drug": justifications for the illegal use of ADHD stimulants. *Subst Use Misuse* **45**(1-2): 31-46.

DeSantis AD, Webb EM, Noar SM (2008). Illicit use of prescription ADHD medications on a college campus: a multimethodological approach. *J Am Coll Health* **57**(3): 315-324.

Engber TM, Koury EJ, Dennis SA, Miller MS, Contreras PC, Bhat RV (1998). Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. *Neurosci Lett* **241**(2-3): 95-98.

Erikson GC, Hager LB, Houseworth C, Dungan J, Petros T, Beckwith BE (1985). The effects of caffeine on memory for word lists. *Physiol Behav* **35**(1): 47-51.

Erman MK, Rosenberg R, For The USMSWSDSG (2007). Modafinil for excessive sleepiness associated with chronic shift work sleep disorder: effects on patient functioning and health-related quality of life. *Prim Care Companion J Clin Psychiatry* **9**(3): 188-194.

Farah MJ, Haimm C, Sankoorikal G, Smith ME, Chatterjee A (2009). When we enhance cognition with Adderall, do we sacrifice creativity? A preliminary study. *Psychopharmacology (Berl)* **202**(1-3): 541-547.

Faraone SV, Biederman J (2002). Efficacy of Adderall for Attention-Deficit/Hyperactivity Disorder: a meta-analysis. *J Atten Disord* **6**(2): 69-75.

Fauchey V, Jaber M, Caron MG, Bloch B, Le Moine C (2000). Differential regulation of the dopamine D1, D2 and D3 receptor gene expression and changes in the phenotype of the striatal neurons in mice lacking the dopamine transporter. *Eur J Neurosci* **12**(1): 19-26.

FDA (2004). FDA Issues Regulation Prohibiting Sale of Dietary Supplements Containing Ephedrine Alkaloids and Reiterates Its Advice That Consumers Stop Using These Products. In: F.D.A. (ed): Rockville, M.D.

FDA (2007). Modafinil (marketed as Provigil): Serious Skin Reactions. In: F.D.A. (ed). *FDA Drug Safety Newsletter*. FDA: Rockville, MD. Vol 1.

FDA (2010). Label and Approval History. Desoxyn. In: F.D.A. (ed). *Approval History NDA 005378*.

Ferguson JT, Linn FV, Nickels MM, Sheets JA, Jr. (1956). Methylphenidate (ritalin) hydrochloride parenteral solution; preliminary report. *J Am Med Assoc* **162**(14): 1303-1304.

Ferraro L, Antonelli T, O'Connor WT, Tanganelli S, Rambert FA, Fuxe K (1997). Modafinil: an antinarcoleptic drug with a different neurochemical profile to d-amphetamine and dopamine uptake blockers. *Biol Psychiatry* **42**(12): 1181-1183.

Ferraro L, Tanganelli S, O'Connor WT, Antonelli T, Rambert F, Fuxe K (1996). The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. *Eur J Pharmacol* **306**(1-3): 33-39.

Findling RL (2008). Evolution of the treatment of attention-deficit/hyperactivity disorder in children: a review. *Clin Ther* **30**(5): 942-957.

Fry JM, For The USMiNMSG, Mitler MM, Guilleminault C, Harsh JR, Hirshkowitz M (1998). Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. US Modafinil in Narcolepsy Multicenter Study Group. *Ann Neurol* **43**(1): 88-97.

Gale AS (1959). Clinical evaluation of methylphenidate (ritalin) hydrochloride in the newborn infant. *J Am Med Assoc* **170**(12): 1408-1409.

Garreau J (2006). A Dose of Genius. 'Smart Pills' Are on the Rise. But Is Taking Them Wise? *The Washington Post*. The Washington Post Company: Washington, D. C.

Goldstein RZ, Leskovjan AC, Hoff AL, Hitzemann R, Bashan F, Khalsa SS*, et al* (2004). Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. *Neuropsychologia* **42**(11): 1447-1458.

Gooding DC, Burroughs S, Boutros NN (2008). Attentional deficits in cocaine-dependent patients: converging behavioral and electrophysiological evidence. *Psychiatry Res* **160**(2): 145-154.

Graham TE (2001). Caffeine and exercise: metabolism, endurance and performance. *Sports Med* **31**(11): 785-807.

Grant S, Contoreggi C, London ED (2000). Drug abusers show impaired performance in a laboratory test of decision making. *Neuropsychologia* **38**(8): 1180-1187.

Greenhill LL, Biederman J, Boellner SW, Rugino TA, Sangal RB, Earl CQ*, et al* (2006). A randomized, double-blind, placebo-controlled study of modafinil film-coated tablets in children and adolescents with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* **45**(5): 503-511.

Gross PT, For The USMiNMSG, Becker PM, Jamieson AO, Jewel CE, Bogan RK*, et al* (2000). Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy: US Modafinil in Narcolepsy Multicenter Study Group. *Neurology* **54**(5): 1166-1175.

Gualtieri CT, Johnson LG (2008). Medications do not necessarily normalize cognition in ADHD patients. *J Atten Disord* **11**(4): 459-469.

Hall KM, Irwin MM, Bowman KA, Frankenberger W, Jewett DC (2005). Illicit use of prescribed stimulant medication among college students. *J Am Coll Health* **53**(4): 167-174.

Hameleers PA, Van Boxtel MP, Hogervorst E, Riedel WJ, Houx PJ, Buntinx F*, et al* (2000). Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. *Hum Psychopharmacol* **15**(8): 573-581.

Hanna JM (1974). Coca Leaf Use in Southern Peru: Some Biosocial Aspects. *American Anthropologist* **76**(2): 281-296.

Haskell CF, Kennedy DO, Milne AL, Wesnes KA, Scholey AB (2008). The effects of L-theanine, caffeine and their combination on cognition and mood. *Biol Psychol* **77**(2): 113-122.

Heal DJ, Cheetham SC, Smith SL (2009). The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety. *Neuropharmacology* **57**(7-8): 608-618.

Henderer JD, Rapuano CJ (2006). Chapter 63. Ocular Pharmacology. In: L. BL, Lazo JS, Parker KL (eds). *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition*. The McGraw-Hill Companies: New York.

Henry JD, Mazur M, Rendell PG (2009). Social-cognitive difficulties in former users of methamphetamine. *Br J Clin Psychol* **48**(Pt 3): 323-327.

Hoff AL, Riordan H, Morris L, Cestaro V, Wieneke M, Alpert R*, et al* (1996). Effects of crack cocaine on neurocognitive function. *Psychiatry Res* **60**(2-3): 167-176.

Homer BD, Solomon TM, Moeller RW, Mascia A, DeRaleau L, Halkitis PN (2008). Methamphetamine abuse and impairment of social functioning: a review of the

underlying neurophysiological causes and behavioral implications. *Psychol Bull* **134**(2): 301-310.

Hyman SE (2005). Addiction: a disease of learning and memory. *Am J Psychiatry* **162**(8): 1414-1422.

Indriati E, Buikstra JE (2001). Coca chewing in prehistoric coastal Peru: dental evidence. *Am J Phys Anthropol* **114**(3): 242-257.

Ivey EP (1958). Methylphenidate hydrochloride therapy after attempted suicide; review of the literature and report of a case. *J Am Med Assoc* **167**(17): 2071-2073.

James RS, Sharp WS, Bastain TM, Lee PP, Walter JM, Czarnolewski M*, et al* (2001). Double-blind, placebo-controlled study of single-dose amphetamine formulations in ADHD. *J Am Acad Child Adolesc Psychiatry* **40**(11): 1268-1276.

Jasinski DR (2000). An evaluation of the abuse potential of modafinil using methylphenidate as a reference. *J Psychopharmacol* **14**(1): 53-60.

Johnson-Kozlow M, Kritz-Silverstein D, Barrett-Connor E, Morton D (2002). Coffee consumption and cognitive function among older adults. *Am J Epidemiol* **156**(9): 842-850.

Jovanovski D, Erb S, Zakzanis KK (2005). Neurocognitive deficits in cocaine users: a quantitative review of the evidence. *J Clin Exp Neuropsychol* **27**(2): 189-204.

Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz JS*, et al* (1997). Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *J Clin Pharmacol* **37**(8): 693-703.

Kautz HD (1957). Methylphenidate hydrochloride. *J Am Med Assoc* **163**(16): 1479-1480.

Kim YT, Lee SW, Kwon DH, Seo JH, Ahn BC, Lee J (2009). Dose-dependent frontal hypometabolism on FDG-PET in methamphetamine abusers. *J Psychiatr Res* **43**(14): 1166-1170.

Kingshott RN, Vennelle M, Coleman EL, Engleman HM, Mackay TW, Douglas NJ (2001). Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of residual excessive daytime sleepiness in the sleep apnea/hypopnea syndrome. *Am J Respir Crit Care Med* **163**(4): 918-923.

Kloner RA, Hale S, Alker K, Rezkalla S (1992). The effects of acute and chronic cocaine use on the heart. *Circulation* **85**(2): 407-419.

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

Korotkova TM, Klyuch BP, Ponomarenko AA, Lin JS, Haas HL, Sergeeva OA (2007). Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors. *Neuropharmacology* **52**(2): 626-633.

Kuczenski R, Segal DS (2002). Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci* **22**(16): 7264-7271.

Kuczenski R, Segal DS (2005). Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse. *Biol Psychiatry* **57**(11): 1391-1396.

Kumar R (2008). Approved and investigational uses of modafinil : an evidence-based review. *Drugs* **68**(13): 1803-1839.

Lason W (2001). Neurochemical and pharmacological aspects of cocaine-induced seizures. *Pol J Pharmacol* **53**(1): 57-60.

Lin JS, Hou Y, Jouvet M (1996). Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-fos immunocytochemistry in the cat. *Proc Natl Acad Sci U S A* **93**(24): 14128-14133.

Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH*, et al* (2006). Modulation of D2R-NR2B interactions in response to cocaine. *Neuron* **52**(5): 897-909.

Loke WH (1988). Effects of caffeine on mood and memory. *Physiol Behav* **44**(3): 367-372.

MacDonald JR, Hill JD, Tarnopolsky MA (2002). Modafinil reduces excessive somnolence and enhances mood in patients with myotonic dystrophy. *Neurology* **59**(12): 1876-1880.

Madras BK, Xie Z, Lin Z, Jassen A, Panas H, Lynch L*, et al* (2006). Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J Pharmacol Exp Ther* **319**(2): 561-569.

Maher B (2008). Poll results: look who's doping. *Nature* **452**(7188): 674-675.

Makris AP, Rush CR, Frederich RC, Taylor AC, Kelly TH (2007). Behavioral and subjective effects of d-amphetamine and modafinil in healthy adults. *Exp Clin Psychopharmacol* **15**(2): 123-133.

Malcolm R, Book SW, Moak D, DeVane L, Czepowicz V (2002). Clinical applications of modafinil in stimulant abusers: low abuse potential. *Am J Addict* **11**(3): 247-249.

McCabe SE, Knight JR, Teter CJ, Wechsler H (2005). Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction* **100**(1): 96-106.

Mednick SC, Cai DJ, Kanady J, Drummond SP (2008). Comparing the benefits of caffeine, naps and placebo on verbal, motor and perceptual memory. *Behav Brain Res* **193**(1): 79-86.

Mehendale SR, Bauer BA, Yuan CS (2004). Ephedra-containing dietary supplements in the US versus ephedra as a Chinese medicine. *Am J Chin Med* **32**(1): 1-10.

Meyer JS, Quenzer LF (2005). *Psychopharmacology: Drugs, the Brain and Behavior*, 1 edn. Sinauer Associates: Sunderland, MA, 555pp.

Mignot E, Nishino S, Guilleminault C, Dement WC (1994). Modafinil binds to the dopamine uptake carrier site with low affinity. *Sleep* **17**(5): 436-437.

Minzenberg MJ, Carter CS (2008). Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology* **33**(7): 1477-1502.

Morgan RE, Crowley JM, Smith RH, LaRoche RB, Dopheide MM (2007). Modafinil improves attention, inhibitory control, and reaction time in healthy, middle-aged rats. *Pharmacol Biochem Behav* **86**(3): 531-541.

Morrow SA, Kaushik T, Zarevics P, Erlanger D, Bear MF, Munschauer FE*, et al* (2009). The effects of L-amphetamine sulfate on cognition in MS patients: results of a randomized controlled trial. *J Neurol* **256**(7): 1095-1102.

Muller U, Steffenhagen N, Regenthal R, Bublak P (2004). Effects of modafinil on working memory processes in humans. *Psychopharmacology (Berl)* **177**(1-2): 161-169.

Muniz R, Brams M, Mao A, McCague K, Pestreich L, Silva R (2008). Efficacy and safety of extended-release dexmethylphenidate compared with d,l-methylphenidate and placebo in the treatment of children with attention-deficit/hyperactivity disorder: a

12-hour laboratory classroom study. *J Child Adolesc Psychopharmacol* **18**(3): 248-256.

Myrick H, Malcolm R, Taylor B, LaRowe S (2004). Modafinil: preclinical, clinical, and post-marketing surveillance--a review of abuse liability issues. *Ann Clin Psychiatry* **16**(2): 101-109.

Nathanson MH (1942). New Adrenalin-Like Compounds: Their Action and Therapeutic Application. *Cal West Med* **57**(5): 301-307.

Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M (2003). Effects of caffeine on human health. *Food Addit Contam* **20**(1): 1-30.

O'Brien CP (2006). Chapter 23. Drug Addiction and Drug Abuse. In: L. BL, Lazo JS, Parker KL (eds). *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition*. The McGraw-Hill Companies.

Oh JS, Lyoo IK, Sung YH, Hwang J, Kim J, Chung A*, et al* (2005). Shape changes of the corpus callosum in abstinent methamphetamine users. *Neurosci Lett* **384**(1-2): 76-81.

Olfson M, Marcus SC, Weissman MM, Jensen PS (2002). National trends in the use of psychotropic medications by children. *J Am Acad Child Adolesc Psychiatry* **41**(5): 514-521.

Ovation Pharmaceuticals I (2007). Medication Guide. Desoxyn. Ovation Pharmaceuticals, Inc.: Deerfield, IL.

Pack AI, Black JE, Schwartz JR, Matheson JK (2001). Modafinil as adjunct therapy for daytime sleepiness in obstructive sleep apnea. *Am J Respir Crit Care Med* **164**(9): 1675-1681.

Pastor PN, Reuben CA (2008). Diagnosed attention deficit hyperactivity disorder and learning disability: United States, 2004-2006. *Vital Health Stat 10*(237): 1-14.

Paulus MP, Hozack NE, Zauscher BE, Frank L, Brown GG, Braff DL*, et al* (2002). Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. *Neuropsychopharmacology* **26**(1): 53-63.

Pereira GS, Mello e Souza T, Vinade ER, Choi H, Rodrigues C, Battastini AM*, et al* (2002). Blockade of adenosine A1 receptors in the posterior cingulate cortex facilitates memory in rats. *Eur J Pharmacol* **437**(3): 151-154.

Pietrzak RH, Mollica CM, Maruff P, Snyder PJ (2006). Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. *Neurosci Biobehav Rev* **30**(8): 1225-1245.

Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA (2007). The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry* **164**(6): 942-948.

Prediger RD, Pamplona FA, Fernandes D, Takahashi RN (2005). Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) -- the spontaneously hypertensive rat (SHR). *Int J Neuropsychopharmacol* **8**(4): 583-594.

Qu WM, Huang ZL, Xu XH, Matsumoto N, Urade Y (2008). Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. *J Neurosci* **28**(34): 8462-8469.

Robertson SD, Matthies HJ, Galli A (2009). A closer look at amphetamine-induced reverse transport and trafficking of the dopamine and norepinephrine transporters. *Mol Neurobiol* **39**(2): 73-80.

Rogers PJ, Dernoncourt C (1998). Regular caffeine consumption: a balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacol Biochem Behav* **59**(4): 1039-1045.

Rogers PJ, Martin J, Smith C, Heatherley SV, Smit HJ (2003). Absence of reinforcing, mood and psychomotor performance effects of caffeine in habitual non-consumers of caffeine. *Psychopharmacology (Berl)* **167**(1): 54-62.

Rogers RD, Robbins TW (2001). Investigating the neurocognitive deficits associated with chronic drug misuse. *Curr Opin Neurobiol* **11**(2): 250-257.

Rump AF, Theisohn M, Klaus W (1995). The pathophysiology of cocaine cardiotoxicity. *Forensic Sci Int* **71**(2): 103-115.

Rush CR, Kelly TH, Hays LR, Baker RW, Wooten AF (2002). Acute behavioral and physiological effects of modafinil in drug abusers. *Behav Pharmacol* **13**(2): 105-115.

Safer DJ, Krager JM (1988). A survey of medication treatment for hyperactive/inattentive students. *JAMA* **260**(15): 2256-2258.

Salo R, Nordahl TE, Buonocore MH, Natsuaki Y, Waters C, Moore CD*, et al* (2009). Cognitive control and white matter callosal microstructure in methamphetamine-dependent subjects: a diffusion tensor imaging study. *Biol Psychiatry* **65**(2): 122-128.

SAMHSA (2008). Results from the 2007 National Survey on Drug Use and Health: National Findings. In: Administration SAaMHS (ed). *NSDUH Series H-34, DHHS Publication No. SMA 08-4343*: Rockville, MD.

Schipani A (2008). Bolivian farmers choose coca or rice. *BBC News*, 18 December 2008 edn: El Chapare, Bolivia.

Schwarzschild MA, Xu K, Oztas E, Petzer JP, Castagnoli K, Castagnoli N, Jr.*, et al* (2003). Neuroprotection by caffeine and more specific A2A receptor antagonists in animal models of Parkinson's disease. *Neurology* **61**(11 Suppl 6): S55-61.

Scott JC, Woods SP, Matt GE, Meyer RA, Heaton RK, Atkinson JH*, et al* (2007). Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychol Rev* **17**(3): 275-297.

Shaffer H (1984). Classics revisited. Über Coca. By Sigmund Freud. *J Subst Abuse Treat* **1**(3): 206-217.

Shuman T, Sage JR, Cai DJ, Anagnostaras S (submitted). Interactions between modafinil and cocaine during the induction and expression of conditioned place preference and locomotor sensitization in mice: implications for addiction.

Shuman T, Wood SC, Anagnostaras SG (2009). Modafinil and memory: effects of modafinil on Morris water maze learning and Pavlovian fear conditioning. *Behav Neurosci* **123**(2): 257-266.

Simon P, Hemet C, Costentin J (1996). Analysis of stimulant locomotor effects of modafinil in various strains of mice and rats. *Fundam Clin Pharmacol* **10**(5): 431-435.

Simon P, Panissaud C, Costentin J (1994). The stimulant effect of modafinil on wakefulness is not associated with an increase in anxiety in mice. A comparison with dexamphetamine. *Psychopharmacology (Berl)* **114**(4): 597-600.

Smit HJ, Rogers PJ (2000). Effects of low doses of caffeine on cognitive performance, mood and thirst in low and higher caffeine consumers. *Psychopharmacology (Berl)* **152**(2): 167-173.

Smith BD, White T, Shapiro R (2007). The Arousal Drug of Choice: Sources and Consumption of Caffeine. In: Smith BD, Gupta U, Gupta BS (eds). *Caffeine and Activation Theory: Effects on Health and Behavior*. CRC Press: Boca Raton, pp 9-42.

Somerville W (1946). Effect of Benzedrine of Fatigue in Soldiers. *Can Med Assoc J* **55**(5): 470-476.

Spencer TJ, Wilens TE, Biederman J, Weisler RH, Read SC, Pratt R (2006). Efficacy and safety of mixed amphetamine salts extended release (Adderall XR) in the management of attention-deficit/hyperactivity disorder in adolescent patients: a 4-week, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* **28**(2): 266-279.

Sulzer D, Sonders MS, Poulsen NW, Galli A (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* **75**(6): 406-433.

Svenningsson P, Nairn AC, Greengard P (2005). DARPP-32 mediates the actions of multiple drugs of abuse. *AAPS J* **7**(2): E353-360.

Swanson JM, Greenhill LL, Lopez FA, Sedillo A, Earl CQ, Jiang JG*, et al* (2006). Modafinil film-coated tablets in children and adolescents with attention-deficit/hyperactivity disorder: results of a randomized, double-blind, placebo-controlled, fixed-dose study followed by abrupt discontinuation. *J Clin Psychiatry* **67**(1): 137-147.

Swanson JM, Lerner M, Williams L (1995). More frequent diagnosis of attention deficit-hyperactivity disorder. *N Engl J Med* **333**(14): 944.

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

Talbot K, Stradling J, Crosby J, Hilton-Jones D (2003). Reduction in excess daytime sleepiness by modafinil in patients with myotonic dystrophy. *Neuromuscul Disord* **13**(5): 357-364.

Terry WS, Phifer B (1986). Caffeine and memory performance on the AVLT. *J Clin Psychol* **42**(6): 860-863.

Teter CJ, McCabe SE, LaGrange K, Cranford JA, Boyd CJ (2006). Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. *Pharmacotherapy* **26**(10): 1501-1510.

Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ (2003). Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacology (Berl)* **165**(3): 260-269.

Tye KM, Tye LD, Cone JJ, Hekkelman EF, Janak PH, Bonci A (2010). Methylphenidate facilitates learning-induced amygdala plasticity. *Nat Neurosci* **13**(4): 475-481.

van Boxtel MP, Schmitt JA, Bosma H, Jolles J (2003). The effects of habitual caffeine use on cognitive change: a longitudinal perspective. *Pharmacol Biochem Behav* **75**(4): 921-927.

van Vliet SA, Jongsma MJ, Vanwersch RA, Olivier B, Philippens IH (2006). Behavioral effects of modafinil in marmoset monkeys. *Psychopharmacology (Berl)* **185**(4): 433-440.

Visser SN, Lesesne CA (2005). Mental Health in the United States: Prevalence of Diagnosis and Medication Treatment for Attention-Deficit/Hyperactivity Disorder--United States, 2003. *MMWR Morb Mortal Wkly Rep* **54**(34): 842-847.

Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F*, et al* (2009). Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. *JAMA* **301**(11): 1148-1154.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ*, et al* (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* **14**(2): 169-177.

Volkow ND, Fowler JS, Wang GJ, Telang F, Logan J, Wong C*, et al* (2008). Methylphenidate decreased the amount of glucose needed by the brain to perform a cognitive task. *PLoS One* **3**(4): e2017.

Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L*, et al* (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci* **21**(2): RC121.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS*, et al* (1998). Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. *Am J Psychiatry* **155**(10): 1325-1331.

Walker-Batson D, Curtis S, Natarajan R, Ford J, Dronkers N, Salmeron E*, et al* (2001). A double-blind, placebo-controlled study of the use of amphetamine in the treatment of aphasia. *Stroke* **32**(9): 2093-2098.

Ward CP, Harsh JR, York KM, Stewart KL, McCoy JG (2004). Modafinil facilitates performance on a delayed nonmatching to position swim task in rats. *Pharmacol Biochem Behav* **78**(4): 735-741.

Waters KA, Burnham KE, O'Connor D, Dawson GR, Dias R (2005). Assessment of modafinil on attentional processes in a five-choice serial reaction time test in the rat. *J Psychopharmacol* **19**(2): 149-158.

Wentz CT, Magavi SS (2009). Caffeine alters proliferation of neuronal precursors in the adult hippocampus. *Neuropharmacology*.

Westfall TC, Westfall DP (2006). Chapter 10. Adrenergic Agonsists and Antagonists. In: L. BL, Lazo JS, Parker KL (eds). *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition*. The McGraw-Hill Companies: New York.

Weyandt LL, Janusis G, Wilson KG, Verdi G, Paquin G, Lopes J*, et al* (2009). Nonmedical prescription stimulant use among a sample of college students: relationship with psychological variables. *J Atten Disord* **13**(3): 284-296.

Wigal S, Swanson JM, Feifel D, Sangal RB, Elia J, Casat CD*, et al* (2004). A double-blind, placebo-controlled trial of dexmethylphenidate hydrochloride and d,l-threo-methylphenidate hydrochloride in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* **43**(11): 1406-1414.

Wigal SB, McGough JJ, McCracken JT, Biederman J, Spencer TJ, Posner KL*, et al* (2005). A laboratory school comparison of mixed amphetamine salts extended release (Adderall XR) and atomoxetine (Strattera) in school-aged children with attention deficit/hyperactivity disorder. *J Atten Disord* **9**(1): 275-289.

Wilens TE, Adler LA, Adams J, Sgambati S, Rotrosen J, Sawtelle R*, et al* (2008). Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. *J Am Acad Child Adolesc Psychiatry* **47**(1): 21-31.

Wilens TE, McBurnett K, Bukstein O, McGough J, Greenhill L, Lerner M*, et al* (2006). Multisite controlled study of OROS methylphenidate in the treatment of adolescents with attention-deficit/hyperactivity disorder. *Arch Pediatr Adolesc Med* **160**(1): 82-90.

Wintzen AR, Lammers GJ, van Dijk JG (2007). Does modafinil enhance activity of patients with myotonic dystrophy?: a double-blind placebo-controlled crossover study. *J Neurol* **254**(1): 26-28.

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001). Dopaminergic role in stimulant-induced wakefulness. *J Neurosci* **21**(5): 1787-1794.

Wood SC, Anagnostaras SG (2009). Memory and psychostimulants: modulation of Pavlovian fear conditioning by amphetamine in C57BL/6 mice. *Psychopharmacology (Berl)* **202**(1-3): 197-206.

Wood SC, Fay J, Sage JR, Anagnostaras SG (2007). Cocaine and Pavlovian fear conditioning: dose-effect analysis. *Behav Brain Res* **176**(2): 244-250.

Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V*, et al* (2009). Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J Pharmacol Exp Ther* **329**(2): 738-746.

Zuvekas SH, Vitiello B, Norquist GS (2006). Recent trends in stimulant medication use among U.S. children. *Am J Psychiatry* **163**(4): 579-585.

Chapter 5, in full, is currently being prepared for submission for publication of the material. Wood, S.C., Sage, J.R., Shuman, T., & Anagnostaras, S.G. The dissertation author was the primary investigator and author of this material.

# GENERAL DISCUSSION

These studies support the idea that stimulant dose is a critical component of the cognitive effects of the drug. Chapter 1 focused on cocaine, examining a variety of doses and their effects on Pavlovian fear conditioning in mice. The lowest dose of cocaine administered enhanced learning, while the highest dose disrupted it. While it is challenging today to run an ethical study on cocaine in humans, free from confounds related to previous stimulant use, these findings are supported by an examination of the history of global human use (see Chapter 5). Many cultures consider regular coca leaf chewing to be an integral part of an arduous lifestyle, without causing physical or psychological disturbances (Grinspoon & Bakalar, 1981; Hanna, 1974). By contrast, Western studies have found evidence of both physical and psychological problems with prolonged cocaine use (Jovanovski, Erb, & Zakzanis, 2005). While it is difficult to directly compare studies from different parts of the world, with drastically different methodologies, data from Chapter 1 indicate that the low amount of cocaine ingested throughout the day may account for the lack of significant problems found in coca leaf chewers, while the high doses taken by Western cocaine users may account for their significant impairments.

Similarly, in Chapter 2, low doses of amphetamine enhanced fear conditioning while moderate-to-high doses disrupted it. This finding is also supported by the literature, with a wealth of studies examining the effects of prolonged exposure to very low doses of amphetamine, and a separate literature examining the effects of prolonged exposure to high doses. The beneficial effects of low dose amphetamine

medication, such as Adderall, are well documented (Ahmann *et al.*, 2001; Faraone & Biederman, 2002; Spencer *et al.*, 2006). Likewise, the deleterious effects of prolonged amphetamine abuse at high doses have also been frequently reported (Chung *et al.*, 2007; Henry *et al.*, 2009; Kim *et al.*, 2009; Nordahl *et al.*, 2003; Scott *et al.*, 2007). However, our study was one of the first, to the best of our knowledge, to examine as wide a range of doses on a basic learning and memory paradigm.

We attempted to generalize our low dose amphetamine findings to another form of learning related to fear conditioning, extinction. Chapter 3 outlined our experiments aimed at enhancing cued fear extinction with low doses of amphetamine. While we administered doses of amphetamine previously found to enhance fear acquisition, we did not find evidence for enhanced extinction after administration of the same doses. This null result is in line with previous research examining the effects of amphetamine on extinction of various fear paradigms, albeit at higher doses (Borowski & Kokkinidis, 1998; Mueller, Olivera-Figueroa, Pine, & Quirk, 2009). Extinction is now commonly recognized as a new form of learning, as opposed to forgetting of previously learned associations, and recent work has uncovered discrete neural pathways responsible for fear conditioning and extinction (Myers & Davis, 2007; Quirk & Mueller, 2008). It is possible that the primary targets of amphetamine are not those critical for extinction, and that amphetamine is not a good candidate for extinction enhancement.

Our conclusions regarding stimulant dosages and learning are based exclusively on fear conditioning. While this paradigm is widely used, the

relationships among its most commonly reported measures are not well understood. For example, the period of freezing after training, post-shock freezing, is either considered to be a conditioned response to the training or is ignored, altogether. We confirmed that post-shock freezing is highly correlated to freezing during the context test, supporting previous findings that indicated it is a measure of contextual fear, not an unconditioned response (Fanselow, 1980). We also found that the tone baseline measure was correlated with tone freezing, creating a confound to the common adjustment made in reporting tone fear: subtracting out baseline freezing from freezing during the tone. This issue has also been taken up by the Fanselow lab at UCLA, who recently proposed a new fear conditioning protocol to help reduce levels of tone baseline freezing (Jacobs *et al.*, 2010). Our data support the need for this new protocol, as a correlation between tone baseline and tone fear indicates that the two measures are not independent of each other. Thus simple subtraction would not eliminate the apparent, intrinsic fear of the alternate tone context, assumedly generalized from the original training session.

Chapter 5 concluded with a review of the literature on a variety of stimulants, summarizing a brief history of use of each substance, as well as its mechanism of action, and the effects of low and high doses. We proposed a continuum of activation that applies generally to stimulants, with low doses of stimulants leading to cognitive enhancement, and high doses leading to cognitive deficits and addiction. This is consistent with the inverted-U shape curve proposed by Yerkes and Dodson in their

report on experiments determining the optimal level of arousal for learning in mice (Yerkes & Dodson, 1908).

Our proposed continuum of activation is in agreement with recent reviews on the topics of cognitive enhancement and ADHD. Current theories regarding the etiology of ADHD point to dysfunction of DA and NE systems throughout the brain, with emphasis on the prefrontal cortex (Biederman & Spencer, 1999; Himelstein, Schulz, Newcorn, & Halperin, 2000). Likewise, as outlined in Chapter 5, the beneficial effects of stimulant medications are mediated through modulating levels of dopamine (DA) and norepinephrine (NE) in the prefrontal cortex. A review of trends in cognitive enhancement hypothesized that an intermediate, optimal level of prefrontal cortex catecholamine concentration exists for best cognitive performance (de Jongh, Bolt, Schermer, & Olivier, 2008). Levels either too high or low impair cognitive performance. It thus follows that the "high performers," whose performance resides near the peak of the inverted U, would experience a detrimental effect from increasing catecholamine levels, while the "low performers," such as those with ADHD, would experience enhancement from an increase in catecholamines. This theory was echoed in another recent review positing that impoverished levels of NE and DA in the prefrontal cortex can lead to fatigue, while excessive levels can lead to stress, with both states leading to suboptimal cognitive performance (Arnsten, 2009). The author pointed to this as the cause for ADHD, as well as for why stimulants help in controlling ADHD symptoms.

This framework could help explain the increasingly widespread use of prescription stimulants by those without ADHD, narcolepsy, or other medical conditions whose first line of treatment is stimulants. Americans have been using stimulants for non-medical, enhancing purposes for the better part of the past century, if not longer, with references to academic doping found in publications as early as the 1940s (Nathanson, 1942). More notoriously, Freud's testament to the wonders of cocaine is forever immortalized in *Über Coca*, originally published in German in 1884 (Shaffer, 1984). Interviews with current college students reveal that stimulants are generally taken to help cram for exams, to stay up all night, or to generally alleviate naturally occurring fatigue (DeSantis & Hane, 2010). In other words, students are using prescription stimulants to attempt to maintain peak cognitive performance when their bodies would naturally be declining toward fatigue and distraction. Similarly, Freud had been "feeling slightly out of sorts from fatigue", but within a few minutes of his first cocaine intake, experienced "sudden exhilaration and feeling of lightness" (Shaffer, 1984). Today's stimulant medications are inexpensive and easy to acquire, in comparison to Freud's time, contributing to the widespread use by students to combat common fatigue and achieve desired cognitive performance.

In summary, dose is a frequently overlooked, yet critical determinant of a stimulant drug's cognitive effects. While there are many common misperceptions surrounding stimulants (e.g., they paradoxically calm children with ADHD, certain drugs are beneficial while others are always detrimental), our data and review of the literature indicate that dose more accurately determines their cognitive and behavioral

effects. Low doses of stimulants can lead to cognitive enhancement, while high doses can lead to cognitive impairment and addiction. This theory is summarized in our continuum of psychostimulant activation, which we hope will provide a framework for further research on the cognitive effects of different doses of stimulants.

# REFERENCES


Abikoff, H., Hechtman, L., Klein, R. G., Weiss, G., Fleiss, K., Etcovitch, J., Cousins, L., Greenfield, B., Martin, D., & Pollack, S. (2004). Symptomatic improvement in children with ADHD treated with long-term methylphenidate and multimodal psychosocial treatment. *J Am Acad Child Adolesc Psychiatry, 43*(7), 802-811.

Agay, N., Yechiam, E., Carmel, Z., & Levkovitz, Y. (2010). Non-specific effects of methylphenidate (Ritalin) on cognitive ability and decision-making of ADHD and healthy adults. *Psychopharmacology (Berl), 210*(4), 511-519.

Ahmann, P. A., Theye, F. W., Berg, R., Linquist, A. J., Van Erem, A. J., & Campbell, L. R. (2001). Placebo-controlled evaluation of amphetamine mixture-dextroamphetamine salts and amphetamine salts (Adderall): efficacy rate and side effects. *Pediatrics, 107*(1), E10.

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci, 19*(3), 1106-1114.

Arnsten, A. F. (2009). Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs, 23 Suppl 1*, 33-41.

Arria, A. M., Caldeira, K. M., O'Grady, K. E., Vincent, K. B., Johnson, E. P., & Wish, E. D. (2008). Nonmedical use of prescription stimulants among college students: associations with attention-deficit-hyperactivity disorder and polydrug use. *Pharmacotherapy, 28*(2), 156-169.

Biederman, J., Melmed, R. D., Patel, A., McBurnett, K., Donahue, J., & Lyne, A. (2008). Long-term, open-label extension study of guanfacine extended release in children and adolescents with ADHD. *CNS Spectr, 13*(12), 1047-1055.

Biederman, J., & Spencer, T. (1999). Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. *Biol Psychiatry, 46*(9), 1234-1242.

Blanchard, R. J., & Blanchard, D. C. (1969). Crouching as an index of fear. *J Comp Physiol Psychol, 67*(3), 370-375.

Borowski, T. B., & Kokkinidis, L. (1998). The effects of cocaine, amphetamine, and the dopamine D1 receptor agonist SKF 38393 on fear extinction as measured with potentiated startle: implications for psychomotor stimulant psychosis. *Behav Neurosci, 112*(4), 952-965.

Bradley, C. (1937). The Behavior of Children Receiving Benzedrine. *Am J Psychiatry*(94), 577-585.

Caldwell, J. (2003). An Overview of the Utility of Stimulants as a Fatigue Countermeasure for Aviators. In H. E. Directorate (Ed.). Brooks, TX.

Chung, A., Lyoo, I. K., Kim, S. J., Hwang, J., Bae, S. C., Sung, Y. H., Sim, M. E., Song, I. C., Kim, J., Chang, K. H., & Renshaw, P. F. (2007). Decreased frontal white-matter integrity in abstinent methamphetamine abusers. *Int J Neuropsychopharmacol, 10*(6), 765-775.

Davidson, M. A. (2008). ADHD in adults: a review of the literature. *J Atten Disord, 11*(6), 628-641.

Davis, M., Ressler, K., Rothbaum, B. O., & Richardson, R. (2006). Effects of D-cycloserine on extinction: translation from preclinical to clinical work. *Biol Psychiatry, 60*(4), 369-375.

de Jongh, R., Bolt, I., Schermer, M., & Olivier, B. (2008). Botox for the brain: enhancement of cognition, mood and pro-social behavior and blunting of unwanted memories. *Neurosci Biobehav Rev, 32*(4), 760-776.

DeSantis, A. D., & Hane, A. C. (2010). "Adderall is definitely not a drug": justifications for the illegal use of ADHD stimulants. *Subst Use Misuse, 45*(1-2), 31-46.

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci, 15*(4), 177-182.

Faraone, S. V., & Biederman, J. (2002). Efficacy of Adderall for Attention-Deficit/Hyperactivity Disorder: a meta-analysis. *J Atten Disord, 6*(2), 69-75.

Faraone, S. V., & Glatt, S. J. (2010). Effects of extended-release guanfacine on ADHD symptoms and sedation-related adverse events in children with ADHD. *J Atten Disord, 13*(5), 532-538.

Faraone, S. V., Wigal, S. B., & Hodgkins, P. (2007). Forecasting three-month outcomes in a laboratory school comparison of mixed amphetamine salts extended release (Adderall XR) and atomoxetine (Strattera) in school-aged children With ADHD. *J Atten Disord, 11*(1), 74-82.

Grinspoon, L., & Bakalar, J. B. (1981). Coca and cocaine as medicines: an historical review. *J Ethnopharmacol, 3*(2-3), 149-159.

Hanna, J. M. (1974). Coca Leaf Use in Southern Peru: Some Biosocial Aspects. *American Anthropologist, 76*(2), 281-296.

Henry, J. D., Mazur, M., & Rendell, P. G. (2009). Social-cognitive difficulties in former users of methamphetamine. *Br J Clin Psychol, 48*(Pt 3), 323-327.

Himelstein, J., Schulz, K. P., Newcorn, J. H., & Halperin, J. M. (2000). The Neurobiology of Attention-Deficit Hyperactivity Disorder. *Frontiers in Bioscience, 5*, d461-478.

Homer, B. D., Solomon, T. M., Moeller, R. W., Mascia, A., DeRaleau, L., & Halkitis, P. N. (2008). Methamphetamine abuse and impairment of social functioning: a review of the underlying neurophysiological causes and behavioral implications. *Psychol Bull, 134*(2), 301-310.

Jacobs, N. S., Cushman, J. D., & Fanselow, M. S. (2010). The accurate measurement of fear memory in Pavlovian conditioning: Resolving the baseline issue. *J Neurosci Methods, 190*(2), 235-239.

Jovanovski, D., Erb, S., & Zakzanis, K. K. (2005). Neurocognitive deficits in cocaine users: a quantitative review of the evidence. *J Clin Exp Neuropsychol, 27*(2), 189-204.

Kim, Y. T., Lee, S. W., Kwon, D. H., Seo, J. H., Ahn, B. C., & Lee, J. (2009). Dose-dependent frontal hypometabolism on FDG-PET in methamphetamine abusers. *J Psychiatr Res, 43*(14), 1166-1170.

Mathew, S. J., Price, R. B., & Charney, D. S. (2008). Recent advances in the neurobiology of anxiety disorders: implications for novel therapeutics. *Am J Med Genet C Semin Med Genet, 148C*(2), 89-98.

McCabe, S. E., Knight, J. R., Teter, C. J., & Wechsler, H. (2005). Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction, 100*(1), 96-106.

Mueller, D., Olivera-Figueroa, L. A., Pine, D. S., & Quirk, G. J. (2009). The effects of yohimbine and amphetamine on fear expression and extinction in rats. *Psychopharmacology (Berl), 204*(4), 599-606.

Muniz, R., Brams, M., Mao, A., McCague, K., Pestreich, L., & Silva, R. (2008). Efficacy and safety of extended-release dexmethylphenidate compared with d,l-methylphenidate and placebo in the treatment of children with attention-deficit/hyperactivity disorder: a 12-hour laboratory classroom study. *J Child Adolesc Psychopharmacol, 18*(3), 248-256.

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Mol Psychiatry, 12*(2), 120-150.

Nathanson, M. H. (1942). New Adrenalin-Like Compounds: Their Action and Therapeutic Application. *Cal West Med, 57*(5), 301-307.

Nordahl, T. E., Salo, R., & Leamon, M. (2003). Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: a review. *J Neuropsychiatry Clin Neurosci, 15*(3), 317-325.

Olfson, M., Marcus, S. C., Weissman, M. M., & Jensen, P. S. (2002). National trends in the use of psychotropic medications by children. *J Am Acad Child Adolesc Psychiatry, 41*(5), 514-521.

Paulus, M. P., Hozack, N. E., Zauscher, B. E., Frank, L., Brown, G. G., Braff, D. L., & Schuckit, M. A. (2002). Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. *Neuropsychopharmacology, 26*(1), 53-63.

Pigeau, R., Naitoh, P., Buguet, A., McCann, C., Baranski, J., Taylor, M., Thompson, M., & Mac, K. I. I. (1995). Modafinil, d-amphetamine and placebo during 64 hours of sustained mental work. I. Effects on mood, fatigue, cognitive performance and body temperature. *J Sleep Res, 4*(4), 212-228.

Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry, 164*(6), 942-948.

Powers, M. B., Smits, J. A., Otto, M. W., Sanders, C., & Emmelkamp, P. M. (2009). Facilitation of fear extinction in phobic participants with a novel cognitive enhancer: a randomized placebo controlled trial of yohimbine augmentation. *J Anxiety Disord, 23*(3), 350-356.

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56-72.

Rapoport, J. L., Buchsbaum, M. S., & Weingartner, H. (1980). Dextroamphetamine: cognitive and behavioral effects in normal and hyperactive boys and normal adult males. *Psychopharmacol Bull, 16*(1), 21-23.

Rapoport, J. L., Buchsbaum, M. S., Zahn, T. P., Weingartner, H., Ludlow, C., & Mikkelsen, E. J. (1978). Dextroamphetamine: cognitive and behavioral effects in normal prepubertal boys. *Science, 199*(4328), 560-563.

Sahakian, B., & Morein-Zamir, S. (2007). Professor's little helper. *Nature, 450*(7173), 1157-1159.

Scott, J. C., Woods, S. P., Matt, G. E., Meyer, R. A., Heaton, R. K., Atkinson, J. H., & Grant, I. (2007). Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychol Rev, 17*(3), 275-297.

Shaffer, H. (1984). Classics revisited. Uber Coca. By Sigmund Freud. *J Subst Abuse Treat, 1*(3), 206-217.

Somerville, W. (1946). The Effect of Benzedrine on Mental or Physical Fatigue in Soldiers. *Can Med Assoc J, 55*(5), 470-476.

Spencer, T. J., Wilens, T. E., Biederman, J., Weisler, R. H., Read, S. C., & Pratt, R. (2006). Efficacy and safety of mixed amphetamine salts extended release (Adderall XR) in the management of attention-deficit/hyperactivity disorder in adolescent patients: a 4-week, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther, 28*(2), 266-279.

Vaidya, C. J., Austin, G., Kirkorian, G., Ridlehuber, H. W., Desmond, J. E., Glover, G. H., & Gabrieli, J. D. (1998). Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. *Proc Natl Acad Sci U S A, 95*(24), 14494-14499.

Vastag, B. (2001). Pay attention: ritalin acts much like cocaine. *JAMA, 286*(8), 905-906.

Visser, S. N., & Lesesne, C. A. (2005). Mental health in the United States. Prevalence of diagnosis and medication treatment for attention-deficit/hyperactivity disorder--United States, 2003. *MMWR Morb Mortal Wkly Rep, 54*(34), 842-847.

Volkow, N. D., Wang, G., Fowler, J. S., Logan, J., Gerasimov, M., Maynard, L., Ding, Y., Gatley, S. J., Gifford, A., & Franceschi, D. (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci, 21*(2), RC121.

Wilens, T. E., Adler, L. A., Adams, J., Sgambati, S., Rotrosen, J., Sawtelle, R., Utzinger, L., & Fusillo, S. (2008). Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. *J Am Acad Child Adolesc Psychiatry, 47*(1), 21-31.

Yerkes, R. M., & Dodson, J. D. (1908). The Relation of Strength of Stimulus to Rapidity of Habit-Formation. *J Comp Neuro Psych, 18*, 459-482.

Zuvekas, S. H., Vitiello, B., & Norquist, G. S. (2006). Recent trends in stimulant medication use among U.S. children. *Am J Psychiatry, 163*(4), 579-585.

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