2009

Effects of early amphetamine exposure on memory

Wendy Ann Holmquist

EFFECTS OF EARLY AMPHETAMINE EXPOSURE ON MEMORY

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

in

Psychology:

General-Experimental Psychology

---

by

Wendy Ann Holmquist

March 2009

EFFECTS OF EARLY AMPHETAMINE EXPOSURE ON MEMORY

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

by

Wendy Ann Holmquist

March 2009

Approved by:

[redacted]

Cynthia Crawford, Chair, Psychology

3/11/09
Date

[redacted]

Sanders McDougall

[redacted]

Allan Butt

ABSTRACT

Exposure to amphetamine during early postnatal development induces long-term reductions in protein kinase A (PKA) activity. Because PKA activity is known to regulate the production of brain derived neurotrophic factor (BDNF), and reductions in BDNF are associated with memory deficits, we hypothesized that early exposure to amphetamine would lead to declines in both BDNF levels and memory performance. Thus in the present study, rat pups were given daily injections of saline or amphetamine (2.5, 5, 10, or 20 mg/kg) on postnatal days 11-20 and spatial learning was assessed using the Morris water maze on postnatal days 28 and 29. In addition, on postnatal day 30 the striatum and hippocampus were removed and levels of BDNF and TrkB (the BDNF receptor) were measured. Contrary to our predictions, rats pretreated with amphetamine did not show a decline in memory performance or have decreased levels of BDNF or TkrB. Male rats, however, treated with the 20 mg/kg amphetamine performed better on the water maze task than saline-treated males or female rats receiving the same dose. Interestingly, female rats had higher densities of TrkB receptors in the hippocampus than males regardless of drug treatment. In conclusion, amphetamine pretreatment did not lead to learning or

memory deficits in adolescent rats, nor did it lead to decreases in BDNF and TrkB levels.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Cynthia Crawford for her guidance, patience, and support while completing this project. Also, I would like to thank my committee members, Dr. Mc Dougall and Dr. Butt for their input and expertise. Additionally, I would like to thank Sergio Diaz Iniguez, Sergios Charntikov, and Dan Mirtoi for their hard work on this project. Lastly, I would like to thank my family for their love, encouragement, and patience throughout this endeavor.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is a developmental disorder characterized by symptoms of inattention, impulsivity, and hyperkinesias (Sagvolden & Seargeant, 1998; Waslick & Greenhill, 1997). Furthermore, children with ADHD are easily distracted, which leads to decreased academic performance and impairments in learning and memory (Waslick & Greenhill, 1997). According to the 1998 National Institutes of Health Consensus Development Conference Statement (National Institutes of Health, 1998), an estimated 3-5% of school-aged children were diagnosed with ADHD. More recent studies have estimated that 7-8.7% of school-aged children meet the diagnostic criteria for ADHD (Bloom & Cohen, 2007; Froehlich, Lanphear, Epstein, Barbaresi, Katusic, & Kahn, 2007).

ADHD is also diagnosed in younger children with an estimated 1-5% of preschool-aged children in the U.S. meeting the diagnostic criteria for this disorder (Connor, 2002; Gillberg, 1986). Because most preschool-aged children exhibit ADHD-like behaviors at some time, the diagnosis is much more difficult to make in this age group (Smidts & Ooosterlaan, 2007).

Neurobiological Theories of Attention Deficit Hyperactivity Disorder

Although the specific cause of ADHD is unknown, there is a body of literature indicating that many of the behavioral symptoms of ADHD have a neurobiological basis and are related to differences in brain structure and/or function. For example, changes in brain function resulting from exposure to teratogens, maternal drug use, and lead poisoning, have all been suspected as possible causes of ADHD (Bellinger & Needleman, 1985; Brown, Coles, Smith, Platzma, Silverstien, Erikson, & Falek, 1991; Hartsough & Lambert, 1985; Needleman, Gunnoe, Leviton, Reed, Peresie, Maher, & Barrett, 1979; Varley, 1984; Zametikin, Nordahl, Gross, King, Semple, et al., 1990). In addition, there is growing evidence that abnormalities in brain metabolism and structure may play key roles in the manifestation of ADHD. Using positron emission tomography, Zametikin and colleagues (1990) found differences in brain activity between ADHD and non-ADHD individuals. Reduced glucose metabolism was found in many parts of the brain, including the cingulate gyrus, right caudate, right hippocampus and right thalamic regions. Furthermore, magnetic resonance imaging has revealed that ADHD individuals have a smaller splenial area of the corpus collosum than non-ADHD

controls (Hill, Yeo, Campbell, Hart, Vigil, & Brooks, 2003). This structural difference may indicate that there are fewer fibers in the corpus collosum available for the activation of frontal areas of the brain. Similarly, the caudate, an area with many connections to the frontal lobes, has been found to be smaller in the left hemisphere in ADHD individuals than in controls (Filipek, Semrud-Clikeman, Steingard, Renshaw, Kennedy, & Biederman, 1997; Hynd, Hern, Novey, Eliopulous, Marshall, Gonzalez, & Voeller, 1993). These differences in structure may lead to a reduction in signaling to the frontal areas of the brain, thus reducing motor behavior inhibition and negatively affecting attention. This idea is further supported by functional magnetic resonance imaging studies that have found differences in frontal striatal circuitry, with reduced activity in the right medial frontal cortex, right inferior prefrontal cortex, and left caudate nucleus of ADHD children (Castellanos, Giedd, Marsh, Hamburger, Valtuzis, et al., 1996; Filipek et al., 1997; Scharchar, Tannock, & Logan, 1993).

There are many theories about the cause of ADHD that are consistent with the aforementioned structural and metabolic abnormalities observed with ADHD. According to one hypothesis, ADHD patients are thought to have

under-activation of the reticular activating system (Sagvolden & Archer, 1989). The reticular activating system is a subcortical structure that extends from the medulla to the midbrain region (Kelly, 1993; Reitan & Wolfson, 1985). This structure is important for maintaining consciousness and attentional states for the whole brain, as the reticular activating system receives input from most sensory systems and projects this information throughout the central nervous system (Reitan & Wolfson, 1985). According to the reticular activating system hypothesis of ADHD, maintenance and/or direction of attention may be impaired due to a lack of stimulation to the higher cortical regions that mediate attention. In the ADHD patient, the reticular activating system may filter too much sensory information, leading to reduced signaling to the cortex resulting in attention deficits (Klove, 1989).

Other research suggests that genetic factors may be involved in the inattention component of ADHD. In a review of eight molecular studies, Swanson, Flodman, Kennedy, Spence, Moyzis, et al. (2000) investigated the hypothesized association between the dopamine transporter 1 gene and dopamine receptor D_4 gene polymorphism and found that the over replication of these genes may alter

activity in the dopamine networks affecting attention. As a result of these polymorphisms, dopamine transporter 1 may be overefficient in dopamine reuptake, while dopamine receptor D_4 may be subsensitive to dopamine. The impact of these polymorphisms may be reduced activity in dopamine pathways involved in attention (Swanson et al., 2000).

In summary, these theories and data imply that the behavioral symptoms of ADHD are the result of under-active dopamine pathways, and insufficient norepinepherine release from the locus coeruleus. Therefore, it is reasonable to hypothesize that psychostimulants such as amphetamine and methylphenidate alleviate ADHD symptoms by increasing extracellular dopamine and norepinepherine levels in many brain areas.

Psychostimulant Treatment for Attention Deficit Hyperactivity Disorder

In the United States, patients diagnosed with ADHD are most often treated initially with a stimulant drug such as methylphenidate or amphetamine (AMPH), whereas in Europe some form of psychosocial intervention is initially tried (Paule, Rowland, Ferguson, Chelonis, Tannock, Swanson, & Castellanos, 2000; Robinson, Sclar, Skaer, & Galin, 2008). Interestingly, in a United States study examining the management of stimulants for pediatric

patients treated for psychological problems, psychosocial interventions and follow-ups were infrequent. In visits where psychostimulants were prescribed, psychosocial intervention was included less than 50% of the time, and in 21% of cases no recommendations for follow-up visits were made (Hoagwood, Jensen, Feil, Vitiello, & Bhatara, 2000).

Although psychosocial interventions such as behavioral modification have some benefits in the treatment of ADHD (Pelham, Wheeler, & Chronis 1998), treatment with stimulant drugs significantly improves behavioral symptoms in 75-90% of ADHD patients (Arnold, 2000, Robinson et al., 2008). Because methylphenidate and AMPH increase the amount of dopamine available in the brain (During, Bean, & Roth, 1992; Castaneda, Levy, Hardy, & Trujillo, 2000), this finding provides further support for the hypothesis that an insufficient amount of dopamine may be responsible for the behavioral symptoms of ADHD (Castaneda et al., 2000; Levy, & Hobbes, 1996).

Psychostimulant Treatment for Preschool-Aged Children

In contrast to school-aged children, few studies have assessed the efficacy and long-term effect of stimulant treatment in preschool-aged children. Currently, a group

called the Preschool Psychopharmacology Working Group is striving toward the development of psychopharmacological guidelines for the treatment of ADHD in preschool-aged children (Gleason, Egger, Emslie, Greenhill, Kowatch, et al., 2007). Despite this lack of information on the efficacy of psychostimulant treatment, a study that examined trends for prescribing psychotropic drugs to preschoolers (ages 2-4) found that over a five-year period (1991-1995) prescriptions for psychotropic drugs, including psychostimulants, increased 3-fold (Zito, Safer, dos Reis, Gardner, Boles, & Lynch, 2000). As the number of preschool-aged children treated with stimulants increases, so do the concerns over the long-term safety of its use. In addition, manufacturers of these drugs do not recommend their use in children under three, however, "off-label" use is common (DSM Pharmaceuticals, 2002; Gleason et al, 2007).

Although there is currently little evidence that methylphenidate has any negative effects on the developing brain, there have been many studies reporting that AMPH drugs can produce long-term neurochemical deficits in adult animals consistent with neurotoxicity (Hotchkiss & Gibb, 1980; Pu & Vorhees, 1993; Ricaurte, Guillery, Seiden, & Moore, 1982; Ricaurte, Seiden, & Schuster, 1984;

Wagner, Ricaurte, Seiden, Schuster, Miller, & Westley, 1980). Because of these findings and current prescription trends, it is imperative that the safety of AMPH treatment in the developing brain be more closely examined.

CHAPTER TWO

DOPAMINE AND ADRENERGIC SYSTEMS

It has been hypothesized that AMPH- and methylphenidate-induced improvements in attention are mediated by the dopamine and noradrenergic systems. The dopamine system of the rat has two primary ascending pathways, the nigrostriatal and mesocorticolimbic (Butler & Hodos, 1996). The nigrostriatal system originates in the A9 area of the substantia nigra and terminates in the neostriatum (i.e., caudate and putamen; Butler & Hodos, 1996). The mesocorticolimbic system extends from the ventral tegmental area to the limbic system (Butler & Hodos, 1996). These pathways are important for the selection, regulation and maintenance of motor functioning (Mason, 1984). The primary noradrenergic system is the dorsal noradrenergic bundle. This pathway originates in the locus coeruleus and projects to the medial forebrain bundle and limbic system (including the hippocampus, amygdala, septum, and anterior olfactory cortex). This system is important in mediating selective attention, the orienting response, and vigilance (Aston-Jones & Bloom, 1981; Aston-Jones, Chiang, & Alexinsky, 1991).

The rate of synthesis of dopamine is dependent on the amount of tyrosine hydroxylase available in the neuron (Walker, 1986). Extracellular tyrosine (absorbed from the diet or synthesized from dietary phenylalanine), is actively transported into all catecholaminergic neurons, where tyrosine hydroxylase converts it to 3,4-dihydroxyphenylalanine (DOPA). DOPA decarboxylase then converts DOPA to dopamine in the cytoplasm, where it is then taken up into vesicles or granules for storage in the nerve terminal of dopamine neurons. In noradrenergic neurons, an additional enzyme, dopamine β-hydroxylase, converts dopamine to norepinepherine. Interestingly, dopamine β-hydroxylase is found in synaptic vesicles, thus dopamine must be transported into the vesicles for norepinepherine to be synthesized (Jasmine & Ohara, 2005). The release of dopamine and norepinepherine occurs via calcium dependent exocytosis and, after being released into the synapse, these neurotransmitters can bind to their respective receptors on the postsynaptic neuron, or autoreceptors on the presynaptic terminal. When autoreceptors are activated, catecholamine release and synthesis is decreased, as tyrosine hydroxylase activity is suppressed (Roth & Nowycky, 1977).

All dopamine and adrenergic receptors are metabotropic receptors (i.e., G-protein-coupled, Gingrich & Caron, 1993; Sibley & Monsma, 1992). Dopamine receptors are divided into two families, D_1-like and D_2-like, with a total of five sub-types. The D_1-like group consists of D_1 and D_5 receptors, while the D_2-like group contains D_2, D_3, and D_4 receptors (Gingrich & Caron, 1993; Sibley & Monsma, 1992). Adrenergic receptors are divided into three families, α_1, α_2, and β, with a total of eight types. The α receptors are subdivided into α_1 and α_2 families. The α_1 family consists of α_{1A}, α_{1B}, and α_{1D}, and the α_2 family includes α_{2A} and α_{2B} receptors. The β family includes β_1, β_2, and β_3 receptors (Bylund, 1992; Rho & Storey, 2001; U'Pritchard & Snyder, 1979).

Dopamine and norepinepherine are primarily inactivated by active reuptake into the presynaptic terminal through the transporter proteins DAT and NET, respectively. In addition, metabolism by monoamine oxidase and catechol-O-methyltransferase also inactivate these neurotransmitters (Costa & Sandler, 1972; Walker, 1986). The two primary dopamine metabolites produced via these enzymatic actions are 3,4-dihydroxyphenylacetaldehyde (DOPAC) and homovanillic acid, while the breakdown of

norepinepherine produces several compounds, including 3-methyoxy-4-hydroxy-phenylglycol and vanillymandelic acid (Costa & Sandler, 1972).

CHAPTER THREE

PSYCHOSTIMULANTS

Mechanisms of Action

AMPH and methylphenidate are catecholaminergic agonists which both increase levels of synaptic dopamine and norepinepherine (Groves, Ryan, Young, & Fisher, 1989; Kuczenski & Segal, 1997). Both psychostimulants rapidly accumulate in the brain following administration (within 1-5 minutes post IV administration, or 15-30 minutes when administered orally), and they are equally efficacious in alleviating behavioral symptoms of ADHD (Markowitz & Patrick, 2001; Wargin, Kilts, Gualtieri, Ellington, Mueller, Kraemer, & Breese, 1983). However, in spite of their similar effects and structure (Markowitz & Patrick, 2001), these drugs do not share the same mechanisms of action. Methylphenidate primarily acts by blocking the reuptake of dopamine and norepinepherine (Russell, deVilliers, Sagvolden, Lamm, & Taljaard, 1998). In contrast, AMPH preferentially releases newly synthesized dopamine and norepinepherine by reversing the action of the dopamine and norepinepherine reuptake pumps (Kuczenski & Segal, 1997; Shore & Dorris, 1975). In addition, AMPH is a weak base that is thought to disrupt the intracellular

pH gradient, thus allowing amphetamine to diffuse into the cell where it can interact with the vesicular membrane transporter (Sulzer, Maidment, & Rayport, 1993). Alkalization of the vesicle then occurs and the neurotransmitter is released into the cell, where it can then leave thru the cell membrane (Sulzer et al., 1993). AMPH also affects serotonergic neurons (Ricaurte, Schuster, & Sieden, 1980) and through this mechanism alters corticosterone secretion and growth hormone release (Cirulli & Laviola, 2000).

Acute and Repeated Effects of Amphetamine in Adult Rats

AMPH is an indirect dopamine agonist, facilitating the release of dopamine into the synapse by reversing the action of the presynaptic re-uptake pumps and by releasing dopamine from storage vesicles (Kuczenski & Segal, 1997; Shore & Dorris, 1975). Thus, acute and repeated treatment with AMPH increases dopamine release in the striatum and nucleus accumbens, as well as inducing other changes in brain neurochemistry. For example, acute treatment with a low to moderate dose (<5 mg/kg) of AMPH increases glucose utilization in the nucleus accumbens, increases dopamine release, and decreases DOPAC, homovanillac acid, and glutamate concentrations (Miele, Mura, Enrico, Esposito,

Serra, et al. 2000; Porrino, Lucignani, Dow-Edwards, & Sokoloff, 1984; Sharp, Zetterstrom, Ljungberg, & Ungerstedt, 1987).

In addition to the neurochemical changes induced by AMPH, AMPH also produces dose-dependent changes in the behavior of rats. At very low doses (<0.1 mg/kg), acute AMPH produces little or no effects on spontaneous behavior (Grilly & Loveland, 2001). However, a moderate dose (~1.0 mg/kg) of AMPH increases locomotor activity, whereas a high dose (~5 mg/kg) produces stereotypic behaviors such as head bobbing, sniffing, gnawing, and licking (Antoniou & Kafetzopoulus, 1991; Porrino et al., 1984). Repeated treatment with AMPH can also produce an augmented behavioral response called behavioral sensitizaton (Leith & Kuczenski, 1981, 1982). Behavioral sensitization can be induced by as little as one drug exposure and can be detected for months after the last amphetamine treatment (Leith & Kuczenski, 1981, 1982).

At higher doses (e.g. 10 mg/kg every two hours for four injections), repeated amphetamine treatment has neurotoxic effects in rodents, including persistent depletions in striatal dopamine, tyrosine hydroxylase activity, striatal dopamine receptor density, and increases in striatal astrogliosis (Hotchkiss & Gibb,

1980; Pu & Vorhees, 1993; Ricaurte, Guillery, Seiden, & Moore, 1982; Ricuarte, Seiden, & Schuster, 1984; Wagner et al., 1980).

CHAPTER FOUR

AMPHETAMINE AND MEMORY

Dopamine and norepinepherine have modulatory roles in memory formation and function. More specifically, dopamine is thought to be associated with reward expectancy, while norepinepherine may be involved in the maintenance of information about the goal, and the rules to achieve that goal (Rossetti, & Carboni, 2005). Therefore, considering the roles of dopamine and norepinepherine in memory, and the effects of AMPH on these neurotransmitters, it is not surprising that AMPH treatment can alter performance on memory tasks.

In adult rats, AMPH treatment has dose-dependent effects on memory that interact with training experience. For example, acute treatment with AMPH within 24 hours post-training can enhance retention on active avoidance, passive avoidance, and discrimination tasks (Evangelista & Isquirdo, 1971; Haycock, van Buskirk, & Gold, 1977; Krivanek, & McGaugh, 1969). When mice were trained for seven days on a passive avoidance task, treatment with 0.3 or 1.0 mg/kg AMPH 24 hours post-training enhanced retention. With six days of training prior to drug treatment, only the 1.0 mg/kg dose of AMPH enhanced

retention. Interestingly, when animals were trained for only four days, a 1.0 mg/kg post-training injection impaired memory performance (Haycock et al., 1977).

Pre-training with acute low to moderate doses (0.5-2.0 mg/kg) of AMPH decreases latencies in trial-dependent learning tasks, and enhances conditioned behaviors on avoidance and discrimination tasks (Haycock et al., 1977). Interestingly, AMPH withdrawn animals (previously treated with escalating doses of AMPH) exhibit enhanced performance in a water maze task and show less interference from prior learning (Russig, Durrer, Yee, Murphy, & Feldon, 2003).

Exposure to neurotoxic doses of AMPH can cause lasting impairments in learning and memory. For example, when rats are given a neurotoxic dosing regime (four injections of 4.0 mg/kg spaced 2 hr apart), impairments are found on an object recognition task when rats were tested one and three weeks post drug treatment, although no impairments in watermaze performance are observed (Schroder, O'Dell, & Marshall, 2003). Interestingly, some recovery occurs over time following neurotoxic AMPH treatment in adult rats. For example, rats treated with four 12.5 mg/kg injections of AMPH spaced 2 hr hours apart, showed impaired performance on a spatial water maze

task when tested 65 days post-injection. However, animals tested at 139 or 237 days post-injection showed no spatial learning impairment (Friedman, Castaneda, & Hodge, 1998).

Working memory deficits occur following 1.0 mg/kg AMPH while 0.3 mg/kg AMPH showed a trend toward improving working and reference memory performance (Blockland, Honig, & Prickaerts, 1998). These findings support the idea that low doses of AMPH may enhance performance on some learning and memory tasks, while higher doses induce neurotoxicity and result in behavioral deficits.

Spatial Learning and Memory

Rats are animals that spontaneously explore and investigate their environment (Renner & Seltzer, 1991). Furthermore, these rodents are experts on spatial relationships and use innate foraging patterns to search for food when hungry (Haig, Rawlins, Olton, Mead, & Taylor, 1983). Because of these innate behaviors, rats are ideal subjects for the study of spatial learning and memory, and factors affecting these processes. In a typical spatial learning task, rats are required to use distal spatial cues such as pictures, doors, light fixtures, and windows to navigate and complete the task. Spatial learning and memory are thought to be dependent on

the integrity of the hippocampus, because rats with lesions to this brain area have trouble learning and remembering this type of task (Milner, Squire, & Kandel, 1998; Morris, Garrud, Rawlins, & O'Keefe, 1982; Whishaw, 1998; Wood, Dudchenko, & Eichenbaum, 1999).

Two commonly used paradigms for assessing spatial learning and memory in rats are the radial arm maze and the Morris water maze. In both of these mazes, animals can use distal visuospatial cues in the room where the maze is located to solve the maze (Hodges, 1996). In the radial arm maze, the goal is for the animal to learn which arms provide a food reward, without entering an arm that has no reward (considered to be an error in working memory), and without entering a previously visited arm (considered to be a spatial reference memory error). The Morris water maze is an open, circular, water tank that is conceptually divided into four quadrants (Morris, 1981). Located in the center of one quadrant of the maze is a submerged escape platform, camouflaged in such a way that the animal cannot see the platform. The task is for the animal to navigate using distal cues to locate the hidden platform efficiently over successive trials.

Although both mazes have been used successfully for assessing spatial learning, working memory, and reference

memory, the Morris water maze has a few advantages. For example, animals do not have to be food or water deprived, odor trails are virtually nonexistent, and motivation to find the escape platform is very high (Hodges, 1996). Interestingly, normal, healthy, rats quickly acquire spatial learning tasks using either the radial arm maze or the Morris water maze (Olton & Samuelson, 1976).

Amphetamine and Spatial Learning

When adult rodents are administered AMPH, deficits are often seen in spatial working and reference memory while the animals are under the influence of the drug (Beatty, Bierley, & Boyd, 1984; Blockland et al., 1998; Bushnell & Levine, 1993). However, rats withdrawn from escalating doses of AMPH show more target zone visits and reduced latency to the former platform location during probe trials (escape platform is removed) in the Morris water maze (Russig et al., 2003). In addition, these animals appeared to overcome prior learning interference more readily than saline-treated controls during a reversal-learning task, where the escape platform is moved to a new location (Russig et al., 2003).

Neurotoxic dosing of methamphetamine (4 injections of 12.5 mg/kg, with 2 hr between injections) impairs spatial

learning and memory when tested 65 days post drug treatment. However, some recovery occurs over time, as spatial learning and memory was not impaired at 79 and 165 days post treatment (Friedman et al., 1998).

Amphetamine and Brain Derived Neurotrophic Factor

Brain derived neurotrophic factor (BDNF) is a neurotrophic factor that is important for the growth, survival, and maintenance of neurons, as well as for types of synaptic plasticity such as long-term potentiation (Bimonte-Nelson, Hunter, Nelson, & Granholm, 2003; Danzer, Crooks, Lo, & McNamara, 2002; Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000). The production of BDNF occurs through the activation of cyclic adenosine monophosphate response element binding protein (CREB). In order for BDNF transcription to occur, CREB must first be phosphorylated by protein kinase A. CREB can then bind to cAMP response element on DNA, resulting in BDNF gene transcription (Deogracias, Espliguero, Iglesias, & Rodriguez-Pena, 2004). Interestingly, BDNF mRNA increases after training on a radial arm maze and/or water maze (Kesslak, So, Choi, Cotman, & Gomez-Pinilla, 1998; Mizuno at el., 2000). In addition, if a reduction in BDNF mRNA and protein levels is induced in the hippocampus, the formation, retention,

and recall of spatial memory is impaired (Mizuno et al., 2000; Mu, Li, Yao, & Zhou, 1999). BDNF and tyrosine kinase receptor (TrkB; the receptor used by BDNF) signaling mediates the effect of N-methyl-D-aspatate receptors in the hippocampus. BDNF and TrkB activity promotes the phosphorylation of N-methyl-D-aspatate receptor subunits, enhancing receptor activity and promoting synaptic plasticity (Levine, Crozier, Black, & Plumer, 1998).

There are a limited number of studies examining the effects of amphetamine on BDNF mRNA or the expression of BDNF proteins. However, in one such study, acute amphetamine treatment (5 mg/kg, IP) in rats increased locomotion and stereotyped behaviors, but did not affect the basal expression of radiolabeled BDNF mRNA or protein immunoreactivity in the forebrain (with the exception of the piriform cortex). However, after repeated treatment with AMPH (5 mg/kg for 5 days), stereotypy was enhanced and BDNF mRNA immunoreactivity was elevated in the amygdala, piriform cortex, and hypothalamus (Meredith, Callen, & Scheuer, 2002). An increase in BDNF levels is not surprising, because infusion with BDNF has neuroprotective properties and can reduce neuronal death induced by methamphetamine (Dluzen, 2004; Matsuzaki, Namikawa, Kiyama, Mori, & Sato, 2004).

In summary, hippocampal BDNF and TrkB are important for spatial learning and memory, and there are limited studies assessing the long-term effects of AMPH treatment on BDNF expression.

CHAPTER FIVE

DOPAMINE SYSTEM DEVELOPMENT

The development of dopamine systems in rats begins at 11-15 days of gestation with the differentiation of dopamine neurons (Lauder & Bloom, 1974). By day 18 of gestation in the rat, dopamine release (Normura, Yotsumoto, & Segawa, 1981), and functional dopamine transport mechanisms have been detected (Yotsumoto & Nomura, 1981). At the day prior to birth, mesencephalon dopamine levels are similar to adult levels (PND 60), while the proencephalon dopamine levels are only 25% of adult level. Interestingly, during the first 4-5 hours after birth, overall brain dopamine levels decrease dramatically, then begin to increase once again (Santana, Rodriguez, Alfonso, & Arevalo, 1992). Dopamine levels in the mesencephalon reach adult-like levels by PD 18, and by four weeks of age nigrostriatal dopamine neurons show an adult-like basal discharge rate, bursting pattern, and conduction velocity (Pitts, Freeman, & Chiodo, 1990). Proencephalic dopamine levels are still relatively low during the early postnatal period, reaching only 45% of the adult level by PD 45, and steadily increasing to adulthood (Santana et al., 1992).

Dopamine receptor development also begins early in prenatal development, as D_1-like and D_2-like receptor binding has been seen as early as gestational day 14 (Jung & Bennett, 1996). Both receptor types then increase steadily in the striatum and nucleus accumbens, with peak receptor expression occurring at PD 28, with binding steadily increasing until adulthood (Jung & Bennett, 1996; Srivasta, Morency, & Mishra, 1992; Tarazi, Tomasini, & Baldsessarini, 1998;). However, in the hippocampus peak D_2-like receptor expression does not occur until PD 35 (Jung & Bennett, 1996; Srivasta, Morency, & Mishra, 1992; Tarazi, Tomasini, & Baldessarini, 1998).

Development of the noradrenergic system in the rat follows a similar bi-phasic pattern. Noradrenergic neurons have been identified as early as gestational day 12 (Morris, Dausse, Devynck, & Myer, 1980). Levels of norepinepherine show a steady rise until the day of birth, when levels drop dramatically, then reach adult levels by the fifth postnatal week (Foote, Bloom, & Aston-Jones, 1983). Noradrenergic receptors also begin to develop early and have been identified by gestational day 16, with binding increasing to approximately adult levels at PD 18-28. As with dopamine receptor development, hippocampal noradrenergic receptors show peak expression

later than other brain areas, at PD 30 (Harden, Wolfe, Sporn, Perkins, & Molinoff, 1977; Hartley & Seeman, 1983; Morris et al., 1980).

Behavioral Effects of Amphetamine in Developing Rats

When AMPH is given acutely to preweanling rats or mice, it can produce an increase in locomotor activity and stereotyped behavior, although to a lesser degree than seen in adult animals (Cirulli, & Laviola; 2000; Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, & McDougall, 2000; Crawford, Zavala, Karper, & McDougall, 2000, Kolta, Scalzo, Ali, & Holson, 1990). In addition, AMPH treatment in developing animals results in smaller changes in dopamine and norepinepherine than in older animals, with 0.1 mg/kg and 1.0 mg/kg AMPH producing a slight increase in dopamine release, followed immediately by a significant decrease in dopamine release (Gazzara, Fisher, & Howard, 1986; Gomes-da-Silva, deMiguel, Fernandez-Ruiz, Summavielle, & Tavares, 2004; Lucot, Wagner, Schuster, & Seiden, 1982).

In contrast to findings with adult animals, repeated treatment with AMPH fails to elicit a long-term sensitized response in very young animals, however, AMPH can produce a short-term sensitized response (line crosses,

stereotyped sniffing, and vertical activity) in this age group. For example, pups treated with 1.0, 2.5, or 5 mg/kg AMPH for 4 consecutive days (beginning at PD 11 or PD 17) show a sensitized response when given a challenge injection of the same dose after two abstinence days. However, animals that were tested following eight abstinence days did not show a sensitized response to the challenge injection (McDougall, Duke, Bolanos, & Crawford, 1994). Considering that the dopamine and norepinepherine systems of rats are not fully developed at the ages used in these experiments, the inability of these animals to exhibit long-term sensitization may be due to the lack of maturation in one or more brain areas.

Interestingly, immature rats do not show the same persistent depletions in striatal dopamine, tyrosine hydroxylase activity, and striatal dopamine receptors that are observed in adult animals after administration of neurotoxic doses of AMPH (Hotchkiss & Gibb, 1980; Wagner et al., 1980; Wagner, Schuster, & Seiden, 1981). In adult animals, prolonged depletion of striatal dopamine by neurotoxic doses of AMPH can be prevented by prior depletion of dopamine stores (Sieden & Schuster, 1985), and by inhibiting dopamine uptake prior to, or shortly after, AMPH treatment (Fuller & Hemrick-Luecke, 1980).

This pattern of results suggests that young animals are more resistant to AMPH-induced neurotoxic damage, perhaps due to the immaturity of the dopamine system and an inability to produce and release large stores of dopamine.

Long-Term Effects of Early Amphetamine Treatment

Repeated administration of AMPH in developing rats causes long-term reductions in striatal and accumbal protein kinase A activity persisting into adulthood (Crawford, Zavala, Karper & McDougall, 2000). AMPH induced reductions in protein kinase A functioning may be a cause for concern in the developing brain, as cyclic adenosine monophosphate dependent protein kinase A pathways are important for many functions, including learning, memory, reward, and addiction (Abel, Nguyen, Barad, Deuel, Kandel, & Bourtchouladze, 1997; Beninger & Miller, 1998; Duffy & Nguyen, 2003; Micheau & Reidel, 1999; Nestler & Aghajanian, 1997). For example, inhibition of protein kinase A disrupts long-lasting (or late phase) long-term potentiation in hippocampal slices, and interferes with memory consolidation in hippocampal dependent memories (Abel et al., 1997; Duffy & Nguyen, 2003). In addition, it has been suggested that protein kinase A plays an important role in the formation of spatial memories

(Mizuno, Yamada, Maekawa, Kuniaki, Seishima, & Nabeshima, 2002). When activated, protein kinase A phosphorylates receptor proteins and gene transcription factors, thereby altering the excitability of neurons (Shobe, 2001). It has been hypothesized that memory is formed when potassium channels are phosphorylated and neurotransmitter release increases (Yao & Wu, 2001).

The finding that AMPH treatment early in development results in a long-term reduction in protein kinase A activity is not consistent with previous studies that have suggested that few, if any, long-term negative consequences result from this type of treatment (Spencer, Beiderman, Harding, O'donnell, Farone, & Willens, 1996). It has been hypothesized that AMPH-induced reductions in protein kinase A activity may occur as a result of changes in dopamine receptors, specifically due to either a downregulation or desensitization of D_1-like receptors, or as a result of an upregulation of D_2-like receptors. Furthermore, it has been found that AMPH treatment can significantly reduce dopamine content in the striatum and nucleus accumbens when compared to saline-treated controls (Ricaurte et al., 1984; Fukumura, Cappon, Pu, Broening, & Vorhees, 1998). Examination of dopamine D_1-like and dopamine D_2-like binding sites revealed that D_1-like

binding sites were unaffected by AMPH treatment, however, a long-term increase in D_2-like binding sites was found. Interestingly, the cyclic adenosine monophosphate dependent protein kinase A pathway is positively coupled to D_1-like receptors and negatively coupled with D_2-like receptors, thus indicating that the upregulation of D_2-like binding sites may be the mechanism by which AMPH-induced reductions in protein kinase A activity occur (Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, & McDougall, 2000; Crawford, Zavala, Karper, & McDougall, 2000).

Ontogeny of Spatial Memory

The development of spatial navigation and memory in rats is associated with hippocampal functioning (Milner et al., 1998; Morris et al., 1982; Whishaw, 1998; Wood et al., 1999). Green and Stanton (1989) noted that age-related differences in spatial learning tasks were very similar to task disassociation seen with hippocampal damage. The hippocampus of a rat grows and develops significantly between PD 0-25. Between PD 0-16, 72% of cells in the denate granule cell layer of the hippocampus are generated, and between PD 11-25, 94% of synapses appear (Altman, Brunner, & Bayer 1973). Green and Stanton

(1989) found that rats as young as 15 days of age show working memory, and that this capacity increases substantially between PD 15-21. Furthermore, 20-day-old rat pups have the capacity to learn a spatial task in the Morris water maze, however, acquisition and retention of this type of task is deficient when compared with mature animals (Brown & Kraemer, 1997).

Assessing Spatial Learning and Memory Deficits in Young Animals

Spatial learning abilities in adult animals are often tested using the Morris water maze, however, there has been some question as to what age young animals can be accurately tested for learning using a water maze. Adams and Jones (1983) used a Y maze water task to answer this question using 18-, 20-, 22-, 28-, and 38-day-old rat pups. They found that 28-day-old animals learned the Y maze task better than the other groups. Significant improvements in the ability of rats to learn the Y maze water task occurred between 20-22 days of age. Brown and Kraemer (1997) examined ontogenetic differences in spatial learning using the Morris water maze, and also found that young animals (older than 20 days) could be successfully tested using this maze. Considering this evidence, along with the hippocampal development data, it seems that a

young rat can be accurately tested for spatial learning abilities using a water maze as early as PD 25.

CHAPTER SIX

THESIS STATEMENT

The purpose of the current study was to determine if rat pups treated with AMPH during a critical time of hippocampal development would exhibit deficits in spatial learning and memory when tested in the Morris water maze during adolescence. In addition, neurochemical assessments were done to determine if this treatment resulted in any long-term changes in hippocampal and striatal BDNF and TrkB. We injected rat pups once per day from PD 11-20 with saline or 2.5, 5, 10, or 20 mg/kg AMPH. These doses of AMPH were chosen because early treatment with similar doses decreased protein kinase A in adult rats (Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, & McDougall, 2000; Crawford, Zavala, Karper, & McDougall, 2000). Animals were then tested beginning on PD 28-29 using the Morris water maze hidden platform paradigm to examine spatial learning and memory. Upon completion of behavioral testing, TrkB immunoblotting and BDNF enzyme-linked immunoassays were performed. All behavioral data were recorded using a computerized video system. During the spatial learning task, acquisition (latency to escape platform), swim path, swim distance, and time spent in the

quadrant where the platform was located were recorded. To test the animal's memory of the platform location, probe trials were conducted in which the platform was removed. During probe trials, time spent in each quadrant, platform site crossings, and time searching was recorded. Following all behavioral testing, hippocampal and striatal BDNF and TrkB levels were assessed.

It was expected animals pretreated with AMPH daily on PD 11-17 to exhibit impairments in spatial learning and retention in the Morris water maze task. This expected impairment included longer latencies to the escape platform during acquisition and less time spent searching in the target quadrant during probe trials when compared to saline-treated controls. In addition, it was predicted that long-term decreases in BDNF and TrkB levels would be seen after early AMPH treatment. This pattern of results was anticipated because previous studies in our laboratory have found long-term reductions in protein kinase A activity following early AMPH treatment. Reductions in protein kinase A activity are important because cyclic adenosine monophosphate dependent pathways are involved in learning and memory. In addition, protein kinase A activity is necessary for the phosphorylation of CREB. CREB phosphorylation regulates BDNF gene transcription,

which is important for synaptic plasticity in learning and memory. Therefore, these predictions were made based on our laboratory's previous findings of reduced protein kinase A activity and the importance of protein kinase A, BDNF, and its receptor TrkB, in learning and memory.

CHAPTER SEVEN

METHODS

Subjects

Subjects were 93 male and female rats (n = 8-10) of Sprague-Dawley descent (Harlan Laboratories) born and raised in the vivarium at California State University, San Bernardino. The rats were housed in the vivarium which was kept on a 12-hr light/dark cycle and maintained at 21-23°C. Rat pups were kept with dams until weaning (PD 25), at which time they were placed in group cages with same sex litter-mates, and remained undisturbed until behavioral testing began. Where possible, one male and one female pup from each litter were assigned to each drug group in order to control for litter effects. Subjects were treated according to the National Institute of Health guidelines for the care and use of laboratory animals ("Principles of Laboratory Animal Care", NIH Publication #85-23).

Drugs and Injections

AMPH was purchased from Sigma Aldrich (St. Louis). AMPH was dissolved in saline and injected intraperitoneally at a volume of 5 ml/kg. On PND 11-20, rat pups were injected once daily with 0.0, 2.5, 5, 10, or

20 mg/kg AMPH. Dams were removed from the litter and placed in separate cages while pups were weighed and injected. The dam was then returned to the home cage.

Apparatus

The Morris water maze consisted of a 122 cm diameter black water tank with a removable transparent platform that was located in one quadrant of the tank during acquisition. During the probe trials the platform was removed. The platform size was 14 cm x 14 cm, and was 1.5-2.0 cm below the surface of the water to conceal its location. Throughout behavioral testing water temperature was kept at 21° ± 1°C.

Pre-Training

Testing began on PND 28 with three pre-training trials. These trials were performed to assess whether the early drug treatment had an effect on swimming ability. In the pre-training trials, a straight swimming channel was placed in the tank. On each trial the rats were placed in the water at one end of the channel and their time to reach a visible platform at the other end of the channel was recorded. When the rat reached the platform it was left on the platform for 15 s before being removed and placed in a heated holding cage for two min.

Acquisition Training

Immediately following pre-training, acquisition trials began. The straight channel was removed and the black platform was placed 1.5-2.0 cm below the surface of the water to obscure its location in the middle of one quadrant in the tank. Start positions were randomly varied among four cardinal start positions along the perimeter, with each animal starting from each position once per day. Rats were placed in the water maze and released facing the wall at the designated starting position for that particular animal. On PD 28 and PD 29, each animal was given two blocks of four trials per day, with 3-4 hr separating blocks. Between blocks, rats were dried and returned to their home cages. In each trial, rats were required to locate the hidden escape platform within the 60 s trial. When the animal reached the platform it remained there for 15 s before being placed in a heated holding cage for the remainder of the 2 min intertrial interval. If the rat failed to find the hidden platform in 60 s, it was placed upon the platform for 15 s, then returned to the holding cage for the remainder of the intertrial interval. After each animal's last trial of the day (on both testing days), rats performed a 1 min probe trial in which the hidden platform was removed. The rat

was placed in the Morris water maze at a designated start point and allowed to search for one min.

Using a video tracking system (Ethno Vision, Noldus Information Technology), swim paths, latency to reach the hidden platform, swim distance and swim speed were recorded during acquisition. For probe trials, time in the quadrant where the platform was previously located, swim distance, and swim speed were recorded.

Tissue Preparation

On the day following behavioral testing, (i.e., PD 30, rats were rapidly decapitated and their hippocampus and striatum were removed. The tissue samples for each animal were divided into two sections and frozen at -80°C until time of assay.

Brain Derived Neurotrophic Factor Enzyme-Linked Immunoassay

BDNF levels in the hippocampus and striatum were examined using the Promega BDNF E_{max} Immunoassay System (Promega, Madison, WI). Briefly, striatal and hippocampal tissue were homongenized in distilled water and sonicated for 15 s. Samples were then centrifuged at 16,000 × *g* for 30 min and resulting supernantant collected. Standard 96-well flat-bottomed Corning ELISA plates were incubated

with carbonate coating buffer containing monoclonal anti-BDNF overnight at 4°C. The following day, the plates were washed three times with TBST buffer. Standard dilutions of BDNF ranging from 0 to 500 pg were performed in duplicate. One hundred µl of the standard dilutions and the tissue samples were added to each well in duplicate and then washed five times with TBST wash buffer. The wells were then incubated with a secondary anti-human BDNF polyclonal antibody (1:500) for 2 h without shaking at room temperature. Plates were washed five times with TBST buffer. Anti-lg Y hoseradish peroxidase conjugate (1:500) was then added to each well and plates were incubated for 1 h with shaking at room temperature. Plates were again washed five times with TBST wash buffer. Finally, plates were developed using 100 µl Promega TMB One Solution and the reaction was stopped at 10 min using 100 µl N HCL. Protein concentrations were determined using the Bio-Rad Protien Assay (Bio-Rad Laboratories, Hercules, CA) based on the method of Bradford (1976), using bovine serum albumin (BSA) as a standard. BDNF levels were reported as ng/mg tissue.

Tyrosine Kinase Receptor (TrkB) Immunoblotting Assay

Hippocampal and striatal homogenates (30 µg/protein) were mixed with 25 µg sample buffer [62.5 mM Tris-HCL (pH 6.8), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.1% w/v bromophenol blue]), boiled for 5 min, centrifuged, and loaded on 15% polyacryamide gels. Rainbow-stained molecular weight markers (Bio-Rad Laboratories) were loaded on each gel. Gels were electrophoresed at 200 V for 2 h. Proteins were transferred to a PVDF membrane (Immuno-Blot, Bio-Rad laboratories) and blocked for 1 h in a solution of 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20. Blots were incubated overnight at room temperature with the primary antibody anti-TrkB (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:1,000. Blots were then washed three times for 10 min in wash buffer (Tris-buffered saline, with 0.1% Tween-20) and incubated in goat anti-rabbit horseradish peroxidase-linked IgG (1:10,000; Pierce Biotechnology, Rockford IL) for 1 h at room temperature. Following this incubation, membranes were washed three times in wash buffer for 10 min and then incubated briefly in peroxidase-chemiluminescence substrate (Super Signal West, Pierce Biotechnology). Immunoreactive bands were

visualized using film-based autoradiography and quantified using a computer-assisted densitometer (model GS-700, Bio-Rad Laboratories). Protein loading and transfer were controlled by stripping (Restore™, Pierce Biotechnology), reblocking, and then reprobing the membranes with a monoclonal antibody to glyceraldehydes 3-phosphate dehydrogenase (GAPDH; Imgenex, San Diego, CA, USA) at a dilution of 1:20,000. Each sample was assayed in duplicate and matched controls were run on each gel.

Statistical Analysis

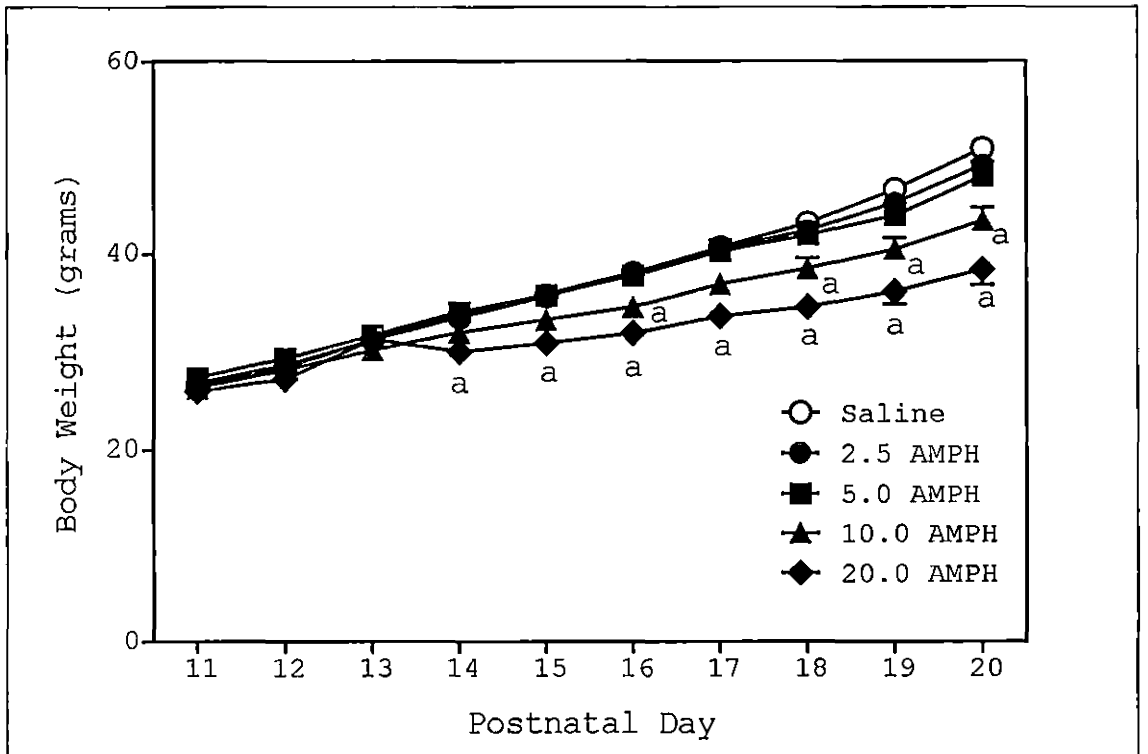
Behavioral data, including latency to escape platform, time spent in target quadrant, swim speed and distance traveled were assessed using separate 5 × 4 × 2 (drug × block × sex) repeated measures ANOVAs for acquisition trials, and separate 5 × 2 × 2 (drug dose × trial × sex) ANOVA's for the probe trials. For the TrkB and BDNF neurochemical assays, separate ANOVAs were used to determine differences between groups. Post hoc analysis were made using Tukey tests ($p < 0.05$).

CHAPTER EIGHT

RESULTS

Weight Data

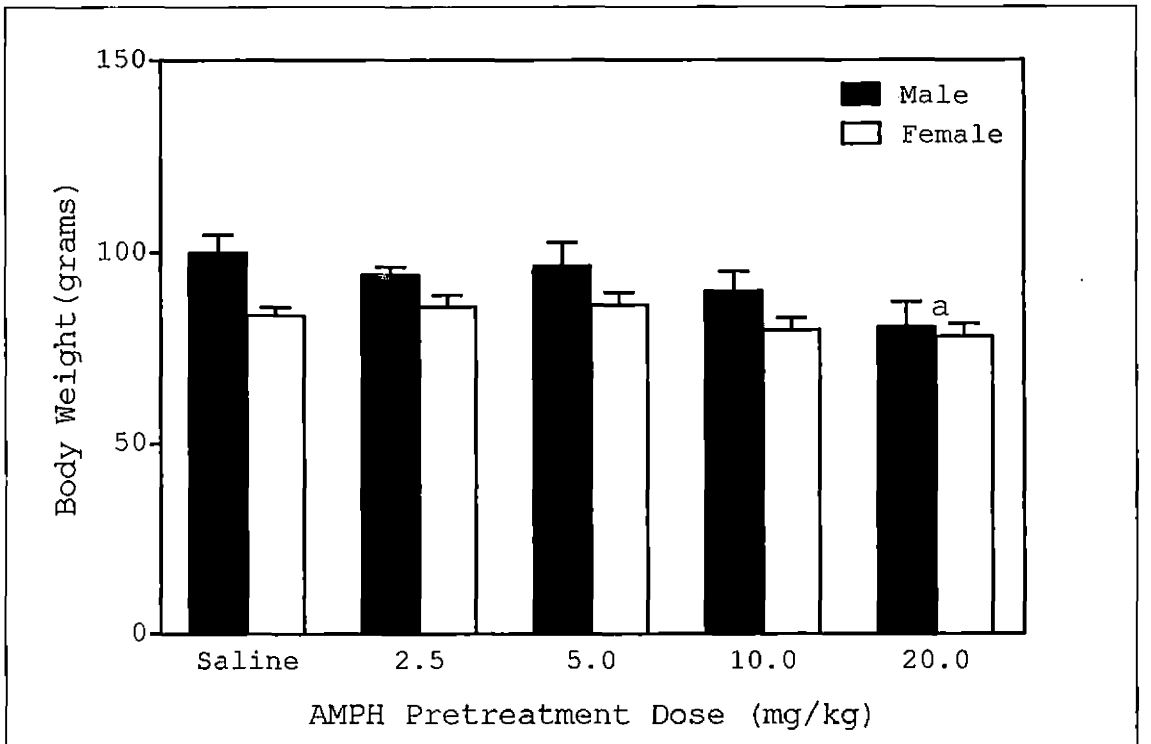
All rats were weighed on injection days (PD 11-20) and on the first test day (PD 28). On PD 11, 12, and 13, there were no significant differences in mean body weight between groups. However, as treatment continued animals receiving 10 or 20 mg/kg AMPH had a lower mean weight than saline animals. Specifically, on PD 14-20, rats receiving 20 mg/kg had a lower mean body weight than the saline group, while those treated with 10 mg/kg had a lower mean body weight than saline animals on PD 16, 18, 19, and 20 [day × drug interaction: $F(36,495) = 11.83$, $p < 0.001$, Tukey Test, $p < .05$, see Figure 1].



[a] Significantly different from saline controls
Data in this figure are collapsed across sex.

Figure 1. Mean Body Weight (Grams) for Male and Female Rats Treated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20

On the first test day (PD 28) animals previously treated with 20 mg/kg still had a lower mean body weight than saline pretreated animals [drug main effect: $F(1,55) = 4.36$, $p < 0.01$, Tukey Test, $p < 0.05$, see Figure 2], whereas the 10 mg/kg group were no longer different than saline animals. In addition, males had higher mean body weight than females on PD 28 [sex main effect: $F(1,56) = 18.84$, $p < 0.001$].



[a] Significantly different from saline controls
Males had significantly higher mean body weights than similarly treated females (main effect of sex)

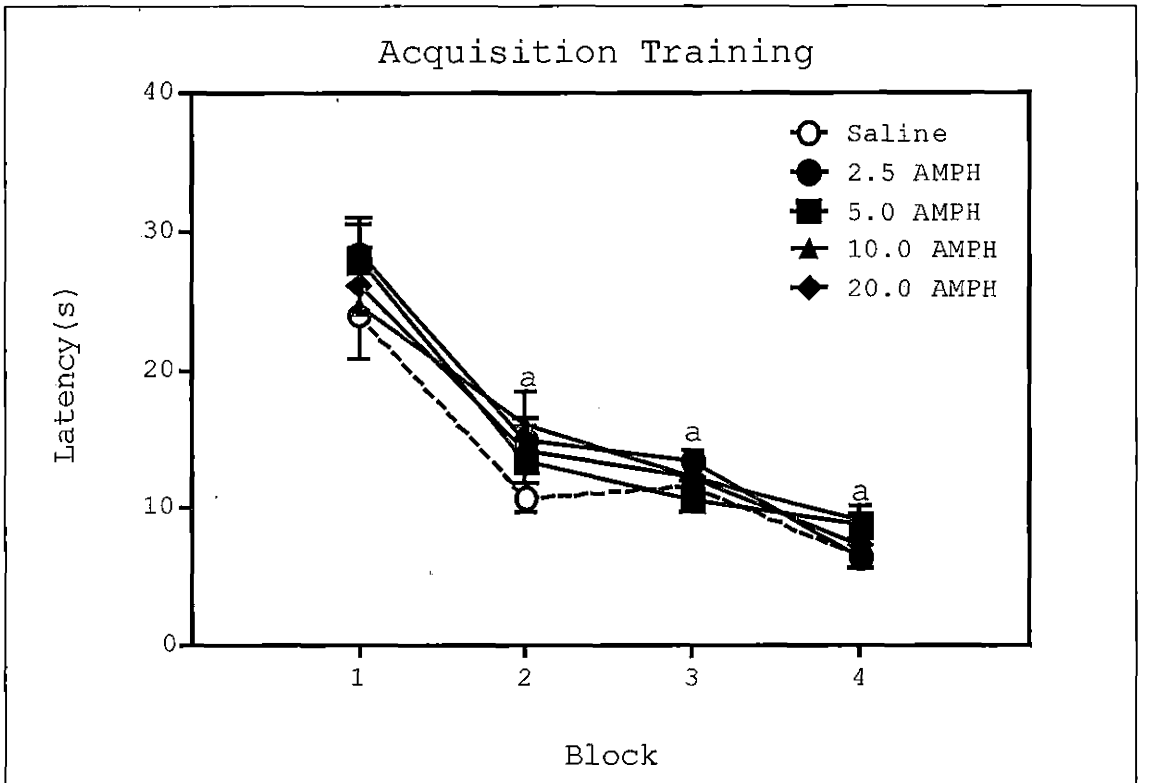
Figure 2. Mean Body Weight (Grams) on PD 28 for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-28

Water Maze Acquisition

All animals swam successfully and located the visible platform during the pretraining session.

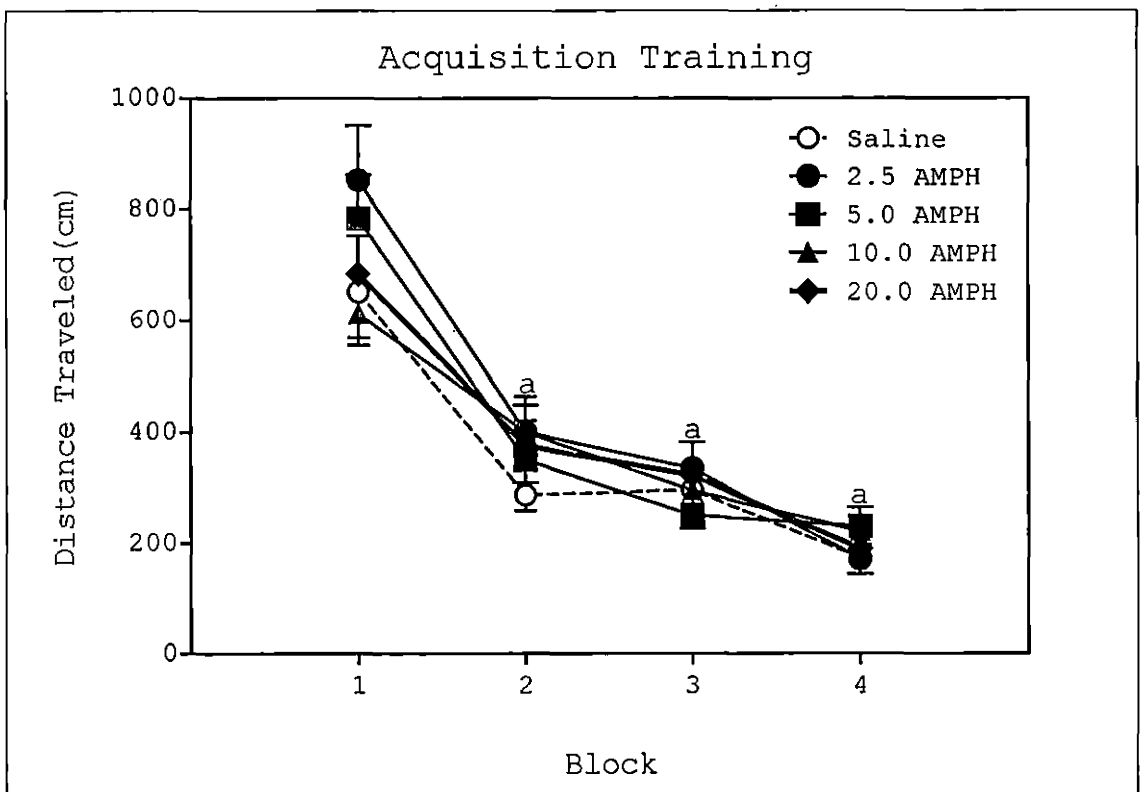
For all groups, latency to reach the hidden platform decreased with each training block regardless of drug treatment or sex [block main effect: $F(3,252) = 106.32$, $p < .05$, Tukey Test, $p < .05$, see Figure 3], indicating

that all groups learned the task in a similar fashion. In addition, swim distance decreased with each block regardless of drug treatment or sex [block main effect: $F(3,249) = 103.19$, $p < .05$, see Figure 4].



[a] Significantly different from block 1.
Data in this figure are collapsed across sex.

Figure 3. Mean Latency (s) to Locate the Escape Platform Across the Four Acquisition Training Blocks for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20



[a] Significantly different from block 1.
Data in this figure are collapsed across sex.

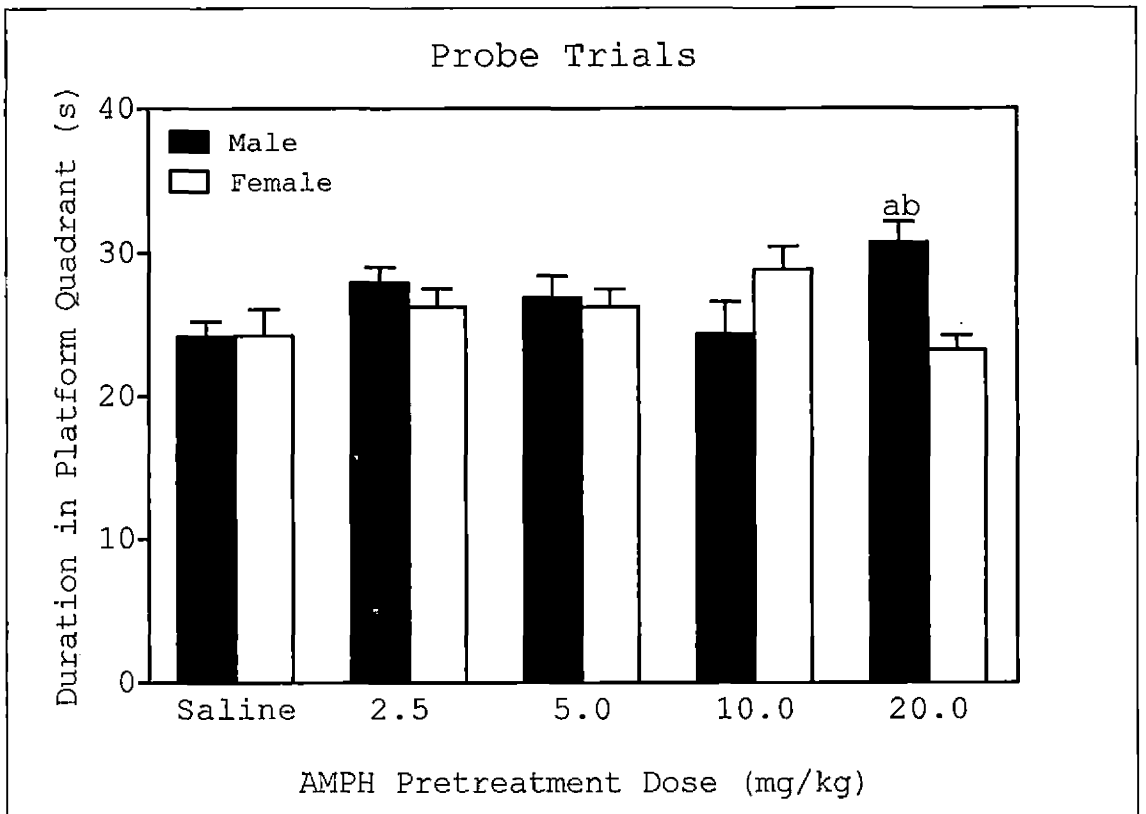
Figure 4. Mean Distance Traveled (cm) to Locate the Escape Platform Across the Four Acquisition Training Blocks for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20

Water Maze Probe Trials

On the probe trials, the high dose of AMPH (20 mg/kg) significantly altered performance, but only in male rats. Specifically, male rats treated with 20 mg/kg AMPH spent more time searching in the target quadrant (the quadrant where the platform had been located previously) than

similarly treated female rats or saline-treated male rats [sex × drug interaction: $F(4,83) = 3.72$, $p < .01$, Tukey Test, $p < 0.05$., see Figure 5]

There was also a significant difference in mean swim velocity between male and female rats, with males swimming faster than females on probe trial 1, but not on probe trial 2 [sex × trial interaction: $F(1,81) = 5.86$, $p < .05$, see Figures 6 & 7]. In addition, on probe trial 1 males swam farther than females regardless of drug group, but on probe trial 2 this difference was no longer present [trial × sex interaction: $F(4,83) = 8.78$, $p < .01$, see Figures 8 & 9].



[a] Significantly different from saline controls
[b] Significantly different from females receiving 20 mg/kg AMPH

Figure 5. Mean Duration in Platform Quadrant(s) across Probe Trials for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20

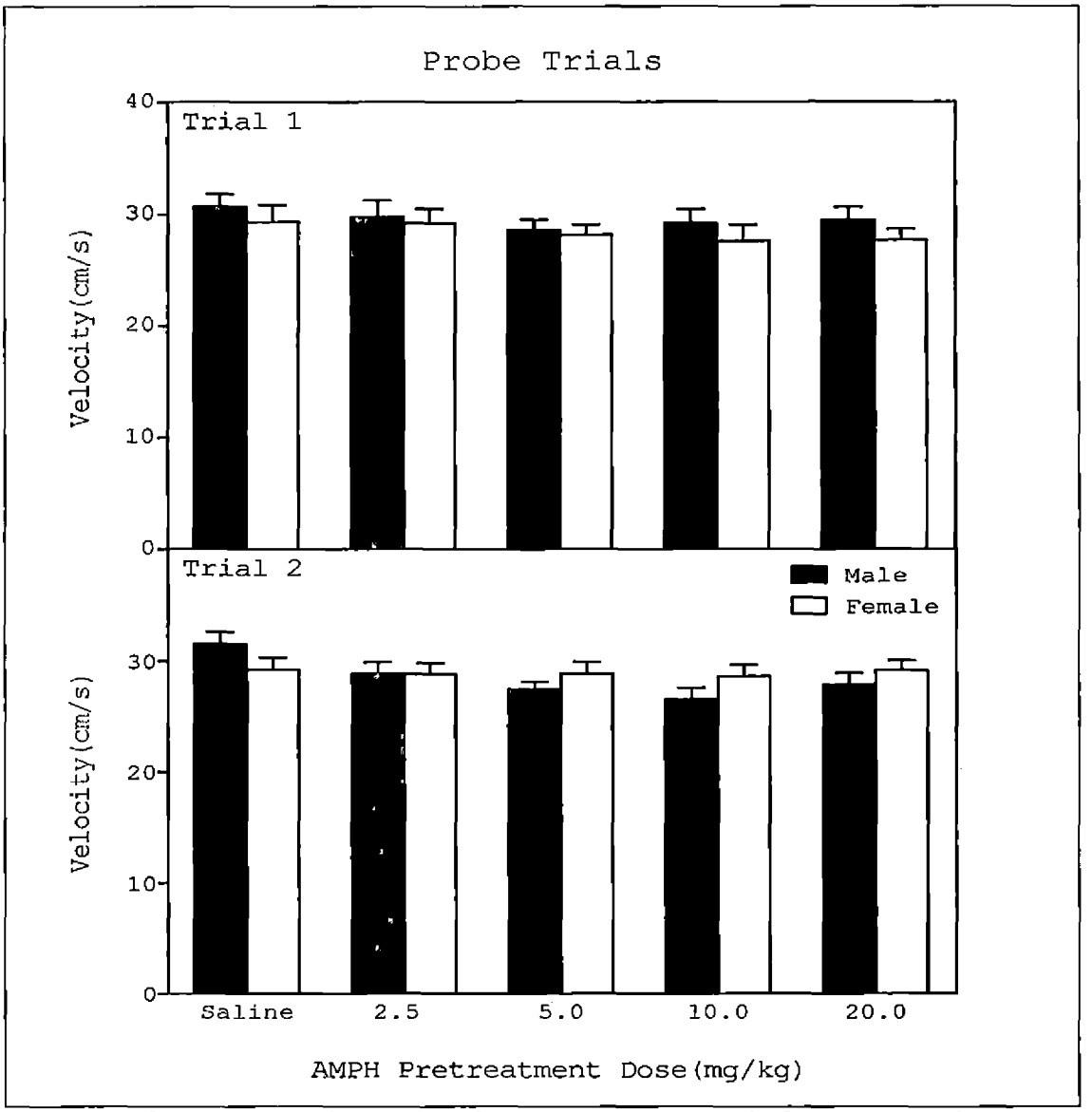
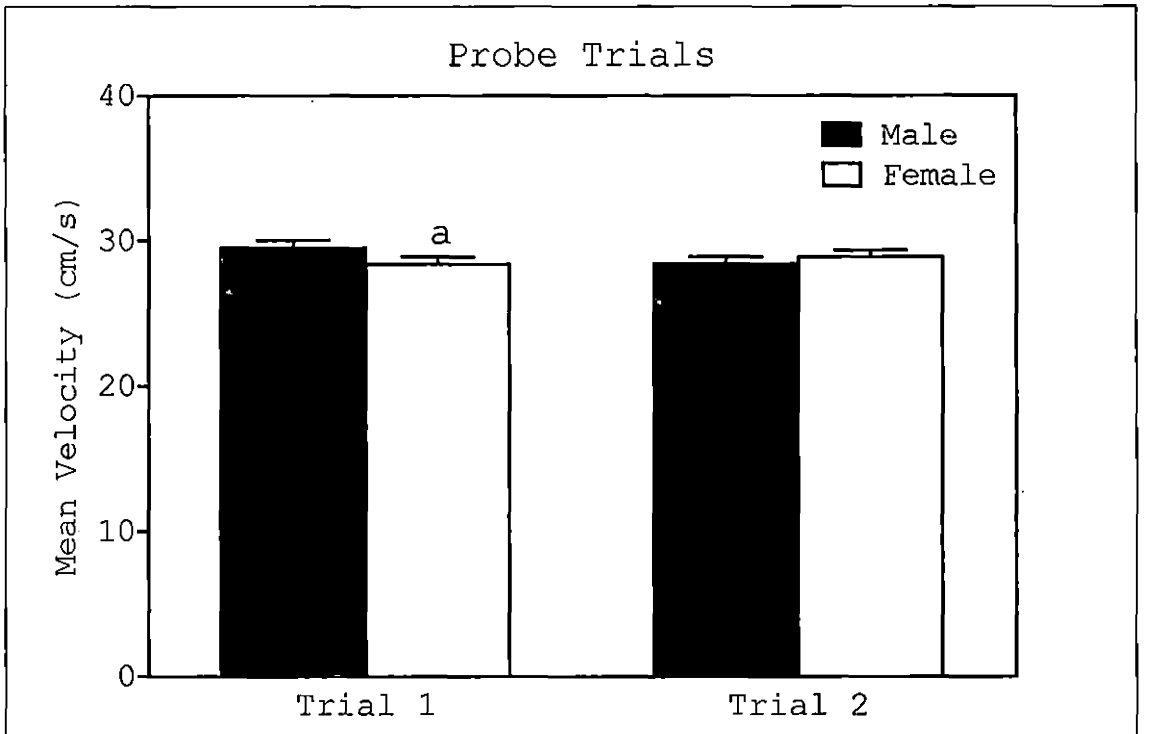


Figure 6. Mean Swim Velocity on Probe Trials One and Two for Male and Female Rats Pretreated with Saline and Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20



[a] Significantly different than males in probe trial 1
Data in this figure are collapsed across amphetamine treatment condition.

Figure 7. Mean Swim Velocity (cm/s) during Probe Trials for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20

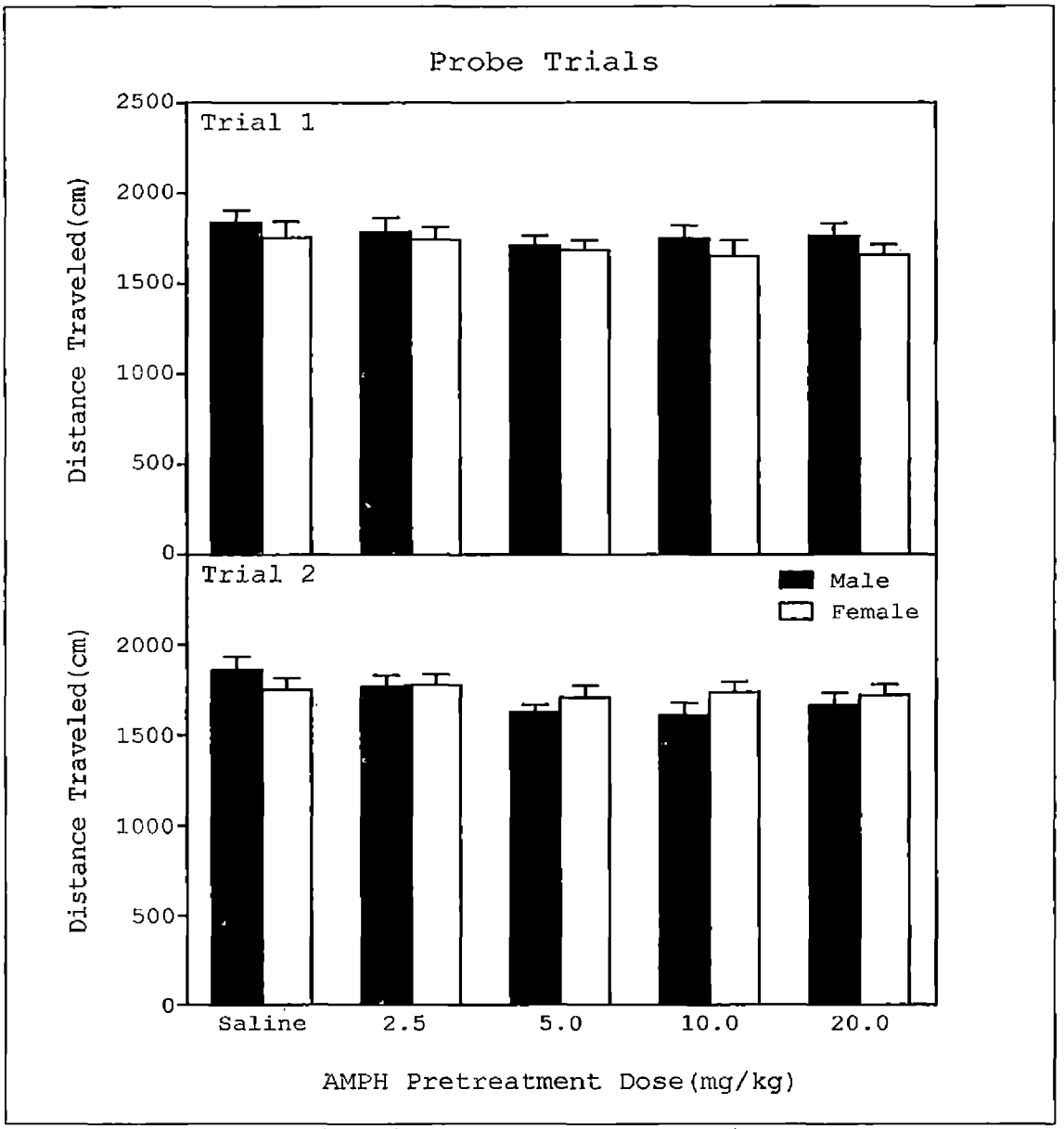
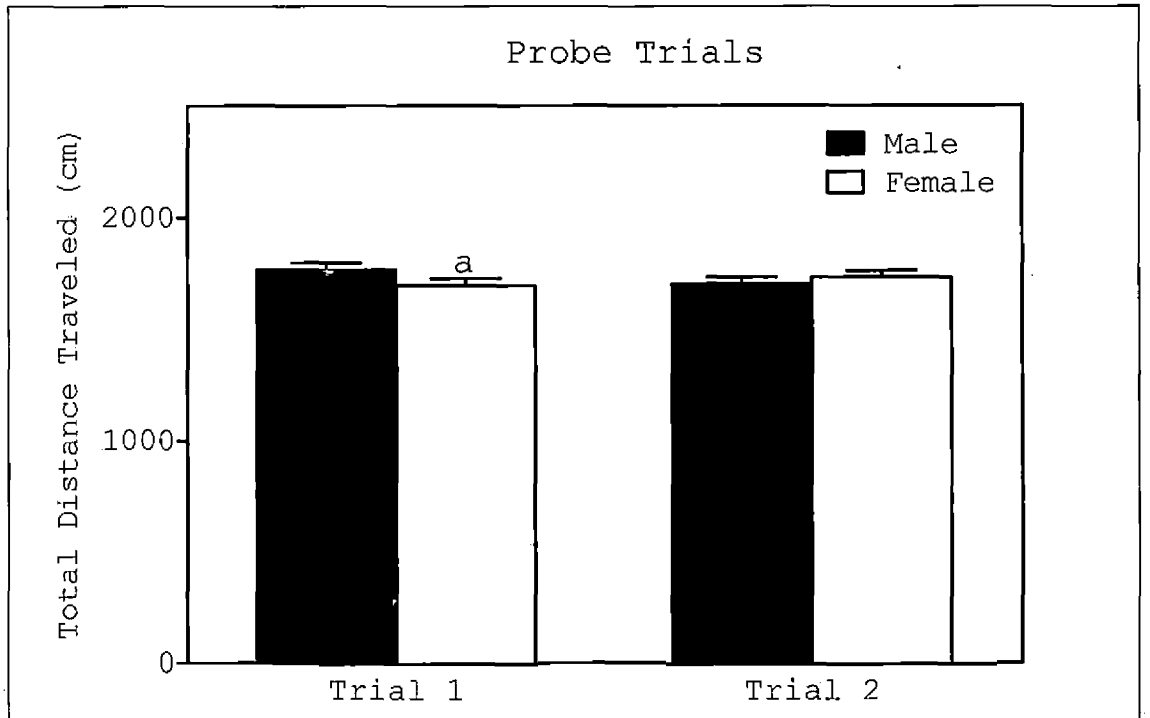


Figure 8. Mean Swim Distance on Probe Trials One and Two for Male and Female Rats Pretreated with Saline and Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20



[a] Significantly different than males rats in probe trial 1. Data in this figure are collapsed across amphetamine treatment condition.

Figure 9. Mean Swim Distance (cm/s) during Probe Trials for Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) from PD 11-20

Tyrosine Kinase Receptor (TrkB) and Brain Derived Neurotropic Factor

Contrary to expectations, when BDNF levels in the striatum and hippocampus were analyzed, no differences between drug treatments or sexes were found. However, when TrkB expression was examined it was found that females had higher densities of TrkB in the hippocampus than males regardless of drug treatment [sex main effect:

$F(1,54) = 5.32$, $p < .05$, see Figures 10 & 11]. In the striatum, there were no significant differences in TrkB densities between sexes or drug groups.

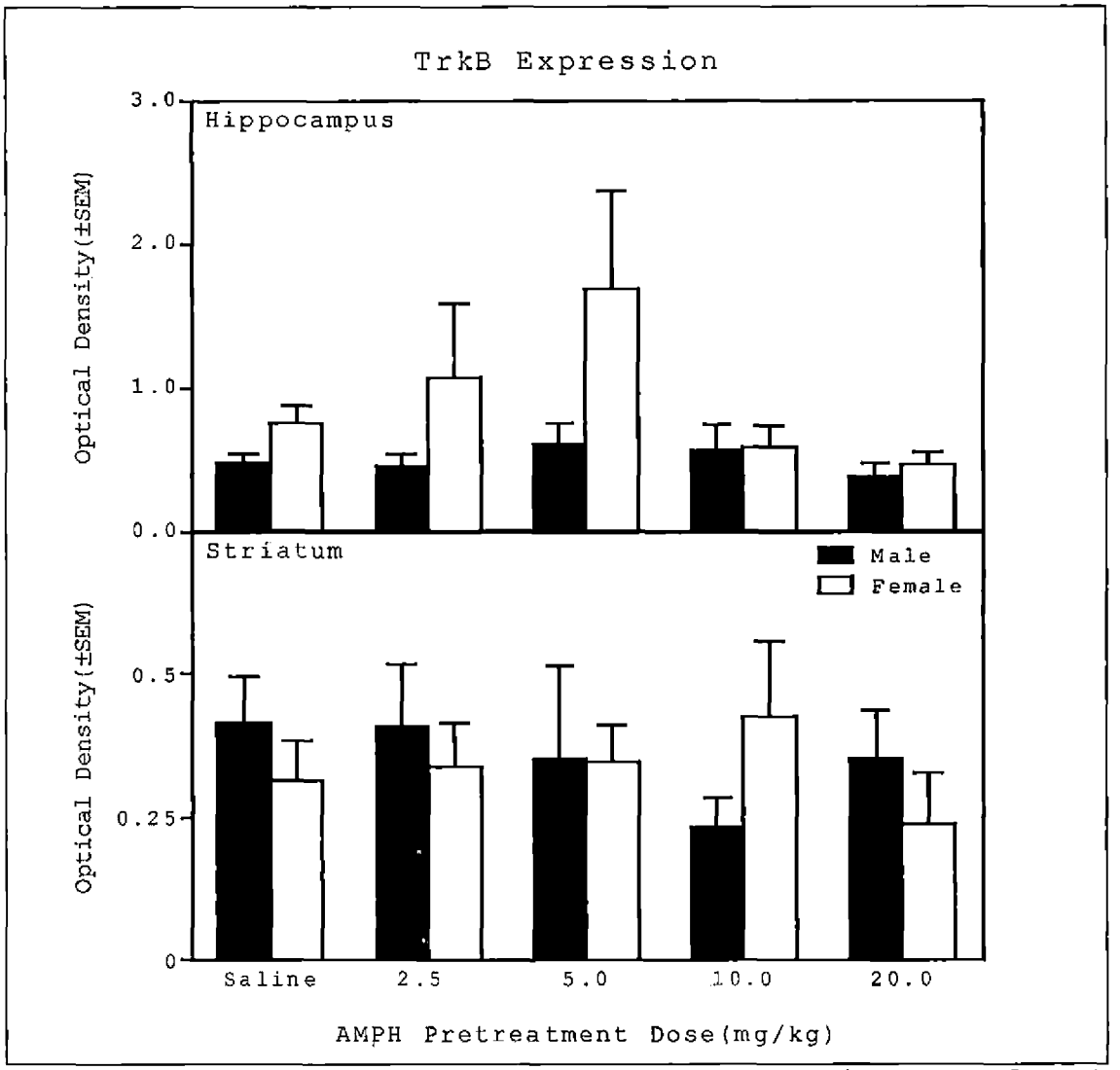
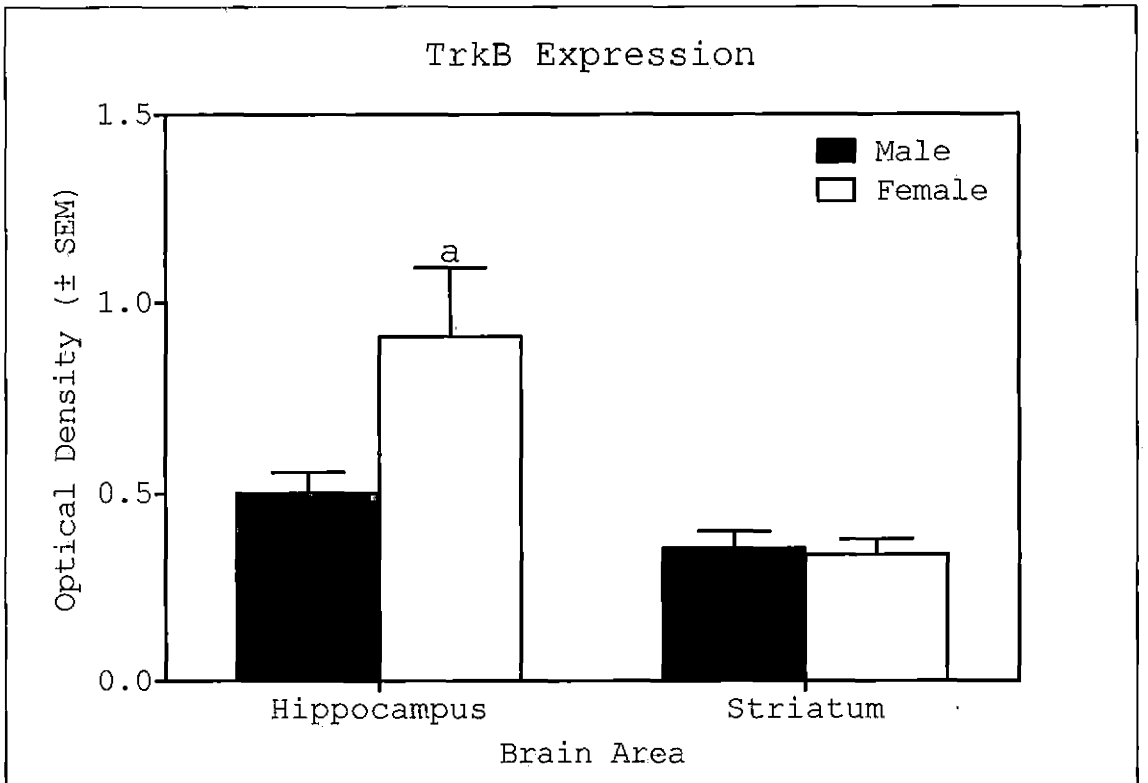


Figure 10. Mean Optical Density (± SEM) of Hippocampal and Striatal Tyrosine Kinase Receptor (TrkB) Expression in Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) on PD 11-20



[a] Significantly different from TrkB expression in the hippocampus of male rats.
Data in this figure are collapsed across amphetamine treatment condition.

Figure 11. Mean Optical Density (± SEM) of Hippocampal and Striatal Tyrosine Kinase Receptor (TrkB) Expression in Male and Female Rats Pretreated with Saline or Amphetamine (2.5, 5.0, 10.0, or 20.0 mg/kg) on PD 11-20 (Collapsed)

CHAPTER NINE

DISCUSSION

Early postnatal exposures to amphetamine and amphetamine analogues have long-lasting neurochemical and behavioral effects in adult rats (Crawford, Choi, Kohutek, Yoshida, & McDougall, 2004; Vorhees, Inman-Wood, Morford, Broeing, Fukumura, & Moran, 2000; Vorhees, Skelton, Williams, 2007; Williams, Morford, Wood, Wallace, Fukumura, Broening, & Vorhees, 2003). Interestingly, it is unknown whether these amphetamine-induced alterations are detectable during other stages of development. Thus, the purpose of the present study was to determine if exposure to AMPH during early postnatal development would alter the behavior and neurochemistry of adolescent rats. Specifically, we treated male and female rat pups from PD 11 to PD 20 with saline or AMPH (2.5, 5.0, 10, or 20 mg/kg) and tested their performance using the Morris water-maze on PD 28 and PD 29. In addition, we measured hippocampal and striatal BDNF levels and TrkB expression. Based on past research we had the following three predictions concerning this study: (1) Animals pretreated with AMPH will exhibit impairments in spatial learning and retention in the Morris water maze, (2) AMPH pretreated

animals will show decreased levels of BDNF compared to saline controls, (3) AMPH pretreated animals will show decreases in TrkB expression compared to saline treated controls.

Effects of Early Amphetamine Treatment on Spatial Learning and Memory

Contrary to what was expected, animals pretreated with AMPH did not have performance deficits in the Morris water maze. During the acquisition phase, the saline and all drug groups (2.5, 5.0, 10.0 and 20 mg/kg AMPH) learned the task in a similar fashion, with no differences between groups in latency to reach the hidden platform. This is consistent with previous findings in young rats that indicate few, if any, long-term negative consequences resulting from amphetamine treatment (Hotchkiss & Gibb, 1980; Spencer et al., 1996; Wagner et al., 1980).

Moreover, during the probe trials male rats pretreated with 20 mg/kg AMPH spent more time searching in the quadrant where the platform was previously located than females receiving the same dose. Males treated with 20 mg/kg AMPH also spent more time searching in the platform quadrant than saline-pretreated controls. This finding was in opposition of our original hypothesis that a high dose of AMPH would impair performance and instead

suggests that pretreatment with 20 mg/kg AMPH can enhance spatial memory, but only in male rats.

One factor that may have contributed to the divergence in probe trial performance is differences in spatial learning strategies used by male and female rats. Previous studies have demonstrated male advantages in spatial learning tasks depending on the paradigm, with males performing better than females when spatial cues and release points are varied (Roof & Stein, 1999). For example, on each of 10 testing days male and female rats were given two trials, with the hidden platform placed in a new, random position each day. Each animal performed an initial trial, followed one hour later by another trial. On the second trial, sex differences were not seen if the release point remained constant. However, if the release point was varied on the subsequent trial, male rats performed better. They also found that female rats could perform as well as males with varied release points as long as the spatial cues in the room remained constant, suggesting that there are differences in the types of spatial cues used by male and female rats (Roof & Stein, 1999). In the current study, it is possible that the type of spatial cues and the movement of the experimenter (a major cue that did not remain constant) were slightly

better suited for male rats as opposed to female rats. Sex differences have also been seen in a study that examined the effects of prior non-spatial training in the Morris water maze on the acquisition and retention of a spatial test in the maze (Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996). Prior non-spatial training in the maze improved acquisition and retention of the spatial task in both sexes. However, in animals that did not receive prior conditioning, males showed better acquisition and retention of the spatial task than females (Perrot-Sinol et al., 1996).

Gondal hormones are an additional factor that may be important to consider regarding sex differences in spatial learning. In a study using meadow voles, male advantages (shorter latencies to find the hidden platform) in spatial learning were found using the Morris water maze (Galea, Kavaliers, Ossenkopp, & Hampson, 1995). Male voles, regardless of current testosterone levels, showed superior spatial learning compared to females with high estradiol levels. This finding suggests that high estradiol levels in female voles may impair performance on this type of spatial task. In contrast, other studies have indicated that high estradiol levels are beneficial on memory because estradiol provides female rats with

neuroprotection from toxic or ischemic insults. Thus, females are less affected than males following chronic stress or chronic AMPH treatment when tested on visual and spatial memory tasks (Bisagno, Bowman, & Luine, 2003; Sandstrom & Rowan, 2007). Although the above studies do not explain the dose dependent male advantage seen in this study, gender specific spatial learning strategies such as the ability of male rats to navigate a spatial task when some cues are inconsistent, and differences in gonadal hormone levels may have contributed to these findings.

Interestingly, in adult animals, repeated low doses of AMPH (0.3 mg/kg) may enhance memory on a spatial task, while a higher dose (1.0 mg/kg) impairs spatial memory performance (Blockland et al., 1998). These findings support the idea that prior exposure to low doses of AMPH may enhance performance on some learning and memory tasks and increase dendritic branching (Li, Kolb, & Robinson, 2003; Robinson, & Kolb, 1997) in adult animals, while higher doses may induce deficits. In addition, it is possible that in young animals, high doses of amphetamine act in a way similar to low doses in adults. This may be due to the immaturity of the dopamine system in young animals. In adults, prolonged depletion of striatal dopamine by neurotoxic doses of methamphetamine can be

prevented by prior depletion of dopamine stores (Sieden & Schuster, 1985) or by inhibiting dopamine uptake prior to or shortly after treatment (Fuller & Hemrick-Luecke, 1980). Young animals given amphetamine early in development (PD 11-21) may not have large stores of dopamine to release, as much of the dopamine system is still developing (Santana et al., 1992).

An alternative explanation for the performance of male rats pretreated with 20 mg/kg AMPH in the water maze, is that the increased duration of time spent in the target quadrant on the probe trials is indicative of a kind of cognitive impairment called perseveration. Perseveration is a cognitive deficit where a response is repeated even though the response is no longer appropriate. While the majority of researchers use increased time in the quadrant that formerly contained the platform as a measure of learning, other researchers have demonstrated that increased time searching in the target quadrant can be a sign of impairment (Hodges, Veizovic, Bray, French, Rashid et al., 2000; Obernier, White, Swartzwelder, Crews, 2002; Van der Zee, Lourenssen, Stanisz, & Diamond, 1995).

In the present study, it is possible that amphetamine pretreatment induced increased activity or sensitivity of dopamine D_2 receptors that lead to perseverative

responding. The basis of this suggestion is that: 1) treatment with quinpirole, (a dopamine D2 agonist) induces perseveration (Ulloa, Nicolini, & Fernandez-Guasti, 2004); 2) decreases in dopamine content lead to increased perseveration (Pioli, Meissner, Sohr, Gross, Bezard, & Bioulac, 2008); and 3) early amphetamine treatment decreases dopamine content and causes an upregulation of dopamine D_2 receptors (Crawford, Zavala, Karper & McDougall, 2000). Additional experiments using different learning task will be necessary to determine whether the increased time in the target quadrant found in the present investigation is the result of increased memory or indicative of cognitive dysfunction.

Effects of Early Amphetamine Treatment on Brain Derived Neurotropic Factor and Tyrosine Kinase Receptor (TrkB) Levels

It was predicted that animals pretreated with AMPH would have a lower density of TrkB and BDNF in the hippocampus and striatum than saline treated animals. This prediction was based on previous finding in our laboratory showing that repeated AMPH treatment reduces protein kinase A activity (Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, & McDougall, 2000; Crawford, Zavala, Karper, & McDougall, 2000 Crawford, Choi, Kohutek,

Yoshida, & McDougall, 2004). Protein kinase A is necessary for the phosphorylation of CREB, which regulates BDNF gene transcription (Deogracias et al., 2004). However, no changes in TrkB or BDNF were induced as a result of drug treatment. Surprisingly, all females in this study, regardless of drug treatment, were found to have elevated TrkB levels compared to males.

BDNF and TrkB activity promotes the phosphorylation of N-methyl-D-aspertate receptor subunits, enhancing receptor activity and promoting synaptic plasticity (Levine et al., 1998). Infusion with BDNF can also have neuroprotective properties and can reduce neuronal death induced by neurotoxins such as methamphetamine and cystocine arabinoside in vitro (Dluzen, 2004; Matsuzaki et al., 2004; Leeds, Leng, Chalecka-Franaszek & Chuang, 2005). For example, BDNF and TrkB levels are increased 24 hours following pretreatment with diethyldithiocarb, a neurotoxic compound that induces apoptic cell death (Micheli, Bova, Laurenzi, Bazzucchi, & Grassi Zucconi, 2006). Furthermore, if TrkB receptor activation is inhibited, the neuroprotective actions of BDNF are attenuated (Leeds et al., 2005). Considering the findings of these past studies, it may not be surprising that female rats were unaffected by AMPH exposure in the

present study. Specifically, because female rats had a higher density of TrKB receptors they may have had more protection against AMPH-induced changes.

The cause for the sex difference in TrkB expression is unknown but may be the result of different levels of circulating estrogens. Other studies have shown a positive relationship between estrogen and BDNF levels in female rats. For example, estrogen replacement in ovariectomized young adult female rats increases BDNF in the hippocampus, cortex, amygdala, and septum (Allen & McCarson, 2005; Signh, Meyer, & Simpkins, 1995; Zhou, Zang, Cohen, & Pandey, 2005). No studies have examined whether estrogen increases BDNF during the preweanling period, however female rats do have larger serum levels of estradiol than male rats on PD 1-21 (Banu, Govindarajulu, & Aruldhas, 2002). In opposition to this hypothesis, however, estrogen treatment in castrated and intact male rat pups (ages PD 4-PD 25) does not affect the expression of TrkB mRNA and protein in the CA1 and CA3 regions of the hippocampus (Sugiyama, Kanba, & Arita, 2003). Unfortunately, this study did not include female rats.

The present study was the first to examine the effect of AMPH on spatial memory and BDNF and TrkB levels during early postnatal development. We predicted that AMPH would

disrupt water maze performance because repeated AMPH (2.5 mg/kg) treatment during the preweanling period induces a long-term decrease in protein kinase A in adult rats (Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, & McDougall, 2000; Crawford, Zavala, Karper, & McDougall, 2000). As mentioned earlier, protein kinase A activity is positively related to BDNF levels and TrkB activity (Deogracias et al., 2004) and reductions in protein kinase A activity can interfere with memory consolidation (Abel et al., 1997; Duffy & Nguyen, 2003; Mizuno et al., 2002). For example, inhibition of protein kinase A activity decreases up-regulation of BDNF mRNA and interferes with the consolidation of a fear memory (Ou & Gean, 2007). In the current study, 2.5, 5.0, 10.0 and 20.0 mg/kg AMPH were administered during the same early postnatal period as the Crawford, Zavala, Karper, Collins, Loring-Meir, Watson, and McDougall (2000) and Crawford, Zavala, Karper, and McDougall (2000) studies, therefore it is likely that protein kinase A activity decreases occurred in these rats as well. However, in the current study protein kinase A was not measured, therefore it is unclear if the changes seen in the two aforementioned studies were present at 30 days of age, or if the deficits in protein kinase A activity take longer to manifest. If

decreases in protein kinase A activity did occur in the current study, the reduction may not have been enough to induce changes in BDNF, TrkB, or water maze performance.

Implications and Conclusions

The current study supports the idea that low doses of AMPH for the treatment of ADHD in young children are relatively safe, as there were no neurochemical deficits found, and behavioral changes were only seen at the highest dose used (20 mg/kg). This dose is much higher than what is recommended in a clinical setting. The American Association of Child and Adolescent Psychiatry Work Group on Quality Issues (2007) developed parameters for the assessment and treatment of children with ADHD, and noted that the maximum dose per day for children 3-5 years old approved by the United States Federal Drug Administration is 40 mg/day (that would be approximately 1.8 mg/kg for a 22 kg child) for Adderall and Dexedrine (American Association of Child and Adolescent Psychiatry Work Group on Quality Issues, 2007), much less than the highest dose used in this study. The recommended starting dose for both of these drugs is only 2.5 mg/kg once or twice daily, and side effects associated with these drugs are most often mild and transient (Ahmann, Theye, Berg,

Linquist, Van Erem, & Campbell, 2001). Considering that the behavioral symptoms of ADHD can be alleviated by psychostimulant treatment in 75-90% of ADHD patients (Arnold, 2000, Robinson et al., 2008), the benefits of ADHD treatment with these drugs appears to outweigh the risks. However, further investigation of the mechanisms of action and long-term safety of stimulant drugs is still needed, especially for younger patients.

REFERENCES

Abel, T., Nguyen, P. V., Barad, M., Deuel, T. A., Kandel, E. R., & Bourtchouladze, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell, 88*, 615-626.

Adams, J., & Jones, S. M. (1983). Age differences in water maze performance and swimming behavior in the rat. *Physiology and Behavior, 33*, 851-855.

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, E10 http//www.pediatrics.org/cgo/content/full/107/1/e10

Allen, A. L., & McCarson, K. E. (2005). Estrogen increases nociception-evoked brain derived neurotrophic factor gene expression in the female rat. *Neuroendocrinology, 81*, 193-199.

Altman, J., Brunner, R. L., & Bayer, S. (1973). The hippocampus and behavioral maturation. *Behavioral Biology, 8*, 557-596.

American Academy of Child and Adolescent Psychiatry Work Group on Quality Issues. (2007). Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*, 894-921.

Antoniou, K., & Kafetzopoulus, E. (1991). A comparative study of the behavioral effects of d-amphetamine and apomorphine in the rat. *Pharmacology, Biochemistry and Behavior, 39*, 61-70.

Arnold, L. E. (2000). Methylphenidate vs. amphetamine: Comparative review. *Journal of Attention Disorders, 3*, 200-211.

Aston-Jones, G., & Bloom, F. E. (1981). Norepinepherine-containing locus coeruleus neurons I behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *Journal of Neuroscience, 1*, 887-900.

Aston-Jones, G., Chiang, C., & Alexinsky, T. (1991). Discharge of noradrenergic locus coeruleus neurons I behaving rats and monkeys suggest a role in vigilance. *Progress in Brain Research, 88*, 501-520.

Banu, S. K., Govindarajulu, P., & Aruldhas, M. M. (2002). Developmental profiles of TSH, sex steroids, and their receptors in the thyroid and their relevance to thyroid growth in immature rats. *Steroids, 67,* 137-144.

Beatty, W. W., Bierly, R. A., & Boyd, J. (1984). Amphetamine disrupts both working and reference memories of rats trained in a radial maze. *Behavioral and Neural Biology, 42,* 160-176.

Bellinger, D. D., & Needleman, H. L. (1985). Prenatal and early postnatal exposure to lead: Developmental effects, correlations, and implications. *Journal of Mental Health, 14,* 78-111.

Beninger, R. J., & Miller, R. (1998). Dopamine D1-like receptors and reward-related incentive learning. *Neuroscience and Biobehavioral Reviews, 22,* 335-345.

Bimonte-Nelson, H. A., Hunter, C. L., Nelson, M. E., & Granholm, A. E. (2003). Frontal cortex BDNF levels correlate with working memory in an animal model of downs syndrome. *Behavioural Brain Research, 139,* 47-57.

Bisagno, V., Bowman, R., & Luine, V. (2003). Functional aspects of estrogen neuroprotection. *Endocrine, 21,* 33-34.

Blockland, A., Honig, W., & Prikaerts, J. (1998). Effects of haloperidol and d-amphetamine on working and reference memory performance in a spatial cone field task. *Behavioral Pharmacology, 9*, 429-436.

Bloom, B., & Cohen, R. A. (2007). Summary health statistics for U.S. children: National Health Interview Survey, 2006. *Vital and Health Statistics, 10*, 1-79.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Annuals of Biochemistry, 72*, 248-254.

Brown, R. T., Coles, C. D., Smith, I. E., Platzma, K. A., Silverstien, J., Erikson, S., & Falek, A. (1991). Effects of prenatal alcohol exposure at school age. II. Attention and behavior. *Neurotoxicology and Teratology, 13*, 369-376.

Brown, R. W., & Kraemer, P. J. (1997). Ontogenetic differences in retention of spatial learning tested with the Morris water maze. *Developmental Psychobiology, 30*, 329-341.

Bushnell, P. J., & Levine, E. D. (1993). Effects of dopaminergic drugs on working and reference memory in rats. *Pharmacology, Biochemistry and Behavior, 45*, 765-776.

Butler, A. B., & Hodos, W. (1996). *Comparative vertebrate neuroanatomy, evolution and adaptation*. New York: Wiley-Liss Inc.

Bylund, D. B. (1992). Subtypes of α_1- and α_2-adrenergic receptors. In G. J. Siegel, B. A. Agranoff, R. W. Albers, S. K. Fisher, & M. D. Uhler (Eds.), *Basic neurochemistry: Molecular, cellular, and medical aspects* (pp. 258). Philadelphia: Lippincott-Raven.

Castaneda, R., Levy, R., Hardy, M., & Trujillo, M. (2000). Long-acting stimulants for the treatment of attention-deficit disorder in cocaine-dependent adults. *Psychiatric services, 51*, 169-171.

Castellanos, F. X., Giedd, J. N., Marsh, W. L., Hamburger, S. D., Valtuzis, A. C., Dickstein, D. P., Sarfatti, S. E., Vauss, J. W., Snell, J. W., Lange, N., Kaysen, D., Krain, A. L., Ritchie, G. F., Rajapakse, J. C., & Rapoport, J. L. (1996). Quantitative brain magnetic resonance imaging in attention-deficit Hyperactivity disorder. *Archives of General Psychiatry, 53*, 607-616.

Cirulli, F., & Laviola, G. (2000). Paradoxical effects of d-amphetamine in infant and adolescent mice: Role of gender and environmental risk factors. *Neuroscience and Biobehavioral Reviews, 24*, 73-84.

Connor, D. F. (2002). Preschool attention deficit hyperactivity disorder: A review of prevalence, diagnosis, neurobiology, and stimulant treatment. *Journal of Developmental Behavioral Pediatrics, 23*, S1-S9.

Costa, E., & Sandler, M. (1972). Monamine oxidase: New vistas. In G. J. Siegel, B. A. Agranoff, R. W. Albers, S. K. Fisher & M. D. Uhler (Eds.), *Basic neurochemistry: Molecular, cellular, and medical aspects* (pp. 247-248). Philadelphia: Lippincott-Raven.

Crawford, C. A., Choi, F. A., Kohutek, J., Yoshida, S. T., & McDougall, S. A. (2004). Changes in PKA activity and $G_{s\alpha}$ and $G_{olf\alpha}$ levels after amphetamine- and cocaine-induced behavioral sensitization. *Synapse, 51*, 241-248.

Crawford, C. A., Zavala, A. R., Karper, P. E., & McDougall, S. A. (2000). Long-term effects of postnatal amphetamine treatment on striatal protein kinase A activity, dopamine D1-like and D2-like binding sites, and dopamine content. *Neurotoxicology and Teratology, 22,* 799-804.

Crawford, C. A., Zavala, A. R., Karper, P. E., Collins, R. L., Loring-Meir, T., Watson, J. B., & McDougall,S. A. (2000). Amphetamine treatment during the preweanling period produces enduring changes in striatal protein kinase A activity. *Pharmacology, Biochemistry and Behavior, 66,* 835-840.

Danzer, S. C., Crooks, K. R., Lo, D. C., & McNamara, J. O. (2002). Increased expression of brain-derived neurotropic factor induces formation of basal dendrites and axonal branching in denate granule cells in hippocampal explant cultures. *The Journal of Neuroscience, 22,* 9754-9763.

Deogracias, R., Espliguero, G., Iglesias, T., Rodriguez-Pena, A. (2004). Expression of the neurotrophin receptor trkB is regulated by the cAMP/CREB pathway in neurons. *Molecular Cellular Neuroscience, 26,* 470-480.

Dluzen, D. E. (2004). The effect of gender and the meurotrophin, BDNF, upon methamphetamine-induced neurotoxicity of the nigrostriatal dopaminergic system in mice. *Neuroscience Letters, 395,* 135-138.

DSM Pharmaceuticals. (2002). *Adderall manufacturer drug bottle insert.* Greenville, NC: DSM Pharmaceuticals Inc.

Duffy, S. N., & Nguyen, P. V. (2003). Postsynaptic application of a peptide inhibitor of camp-dependent protein kinase blocks expression of long-lasting synaptic potentiation in hippocampal neurons. *The Journal of Neuroscience, 23,* 1142-1150.

During, M. J., Bean, A. J., & Roth, R. H. (1992). Effects of CNS stimulants on the in vivo release of the colocalized transmitters, dopamine and neurotensin, from rat prefrontal cortex. *Neuroscience Letters, 140,* 129-133.

Evangelista, A. M., & Izquierdo, I. (1971). The effects of pre and post trial amphetamine on avoidance response of rats. *Psychopharmacologia, 20,* 42-47.

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing attention deficit hyperactivity disorder and normal controls. *Neurology, 48*, 589-601.

Foote, S. L., Bloom, F. E., & Aston-Jones, G. (1983). New evidence of anatomical and physiological specificity. *Physiology Review, 63*, 844-914.

Friedman, S. D., Castaneda, E., & Hodge, G. K. (1998). Long-term monamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacology, Biochemistry and Behavior, 61*, 35-44.

Froehlich, T. E., Lanphear, B. P., Epstein, J. N., Barbaresi, W. J., Katusic, S. K., & Kahn, R. S. (2007). Prevalence, recognition, and treatment of attention-deficit/hyperactivity disorder in a national sample of U.S. children. *Achieves of Pediatric & Adolescent Medicine, 161*, 857-864.

Fukumura, M., Cappon, G. D., Pu, C., Broening, H. W., & Vorhees, C. V. (1998). A single dose model of methamphetamine-induced neurotoxicity in rats: Effects on neostriatal monoamines and glial figrillary acidic protein. *Brain Research, 806*, 1-7.

Fuller, R. W., & Hemrick-Luecke, S. K. (1980). Long lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. *Science, 209,* 305-307.

Galea, L. A., Kavaliers, M., Ossenkopp, K. P., Hampson, E. (1995). Gonadal hormone levels and spatial learning performance in the Morris water maze in male and female meadow voles, Microtus pennsylvanicus. *Hormones and Behavior, 29,* 106-125.

Gazzara, R. A., Fisher, R. S., & Howard, S. G. (1986). The ontogeny of amphetamine-induced dopamine release in the caudate-putamen of the rat. *Brain Research, 393,* 213-220.

Gillberg, C. (1986). Attention deficit disorder: diagnosis, prevalence, management, and outcome. *Pediatrician, 13,* 108-118.

Gingrich, J. A., & Caron, M. G. (1993). Recent advances in the molecular biology of dopamine receptors. In G. J. Siegel, B. A. Agranoff, R. W. Albers, S. K. Fisher, & M. D. Uhler (Eds.), *Basic neurochemistry: Molecular, cellular, and medical aspects* (pp. 254). Philadelphia: Lippincott-Raven.

Gleason, M. M., Egger, H. L., Emslie, G. J., Greenhill, L. L., Kowatch, R. A., Lieberman, A. F., Luby, J. L., Owens, J., Scahill, L. D., Scheeringa, M. S., Stafford, B., Wise, B., & Zeanah, C. H. (2007). Psychopharmacological treatment for very young children: Contexts and guidelines. *Journal of the American Academy of Child and Adolescent Psychiatry, 46,* 1532-1572.

Gomes-da-Silva, J, de Miguel, R., Fernandez-Ruiz, J., Summavielle, T., & Tavares, M. A. (2004). Effects of neonatal exposure to methamphetamine: Catecholamine levels in brain areas of the developing rat. *Annuals of the New York Academy of Science, 1025,* 602-611.

Green, R. J., & Stanton, M. E. (1989). Differential ontogeny of working memory and reference memory in the rat. *Behavioral Neuroscience, 103,* 98-105.

Grilly, D. M., & Loveland, A. (2001). What is a low dose of d-amphetamine for inducing behavioral effects in laboratory rats? *Psychopharmacology, 153,* 155-169.

Groves, P. M., Ryan, L. J., Young, D. M., & Fisher, S. J. (1989). Neuronal actions of amphetamine in the rat brain. *NIDA Research Monographs,* 94, 127-145.

Haig, K. A., Rawlins, J. N. P., Olton, D. S., Mead, A., & Taylor, B. (1983). Food searching strategies of rats: Variables affecting the relative strength of stay and shift strategies. *Journal of Experimental Psychology: Animal Behavior Processes, 9*, 337-348.

Harden, T. K., Wolfe, B. B., Sporn, J. R., Perkins, J. D., & Molinoff, P. B. (1977). Ontogeny of β-adrenergic receptors in rat cerebral cortex. *Brain Research, 125*, 99-108.

Hartley, E. J., & Seeman, P. (1983). Development of receptors for dopamine and noradrenaline in the rat brain. *European Journal of Pharacology, 91*, 391-397.

Hartsough, C. S., & Lambert, N. M. (1985). Medical factors in hyperactive and normal children: Prenatal, developmental, and health history findings. *American Journal of Orthopsychiatry, 55*, 190-201.

Haycock, J. W., van Buskirk, R., & Gold, P. E. (1977). Effects on retention of post-training amphetamine injections in mice: Interaction with pre-training experience. *Psychopharmacology, 54*, 21-24.

Hill, D. E., Yeo, R. A., Campbell, R. A., Hart, B., Vigil, J., &Brooks, W. (2003). Magnetic resonance imaging correlates of attention-deficit/hyperactivity disorder in children. *Neuropsychology, 17*, 496-506.

Hoagwood, K., Jensen, P. S., Feil, M., Vitello, B., & Bhatara, V. S. (2000). Medication management of stimulants in pediatric practice settings: A national perspective. *Journal of Developmental Pediatrics, 21*(5), 322-331.

Hodges, H. (1996). Maze procedures: The radial-arm and water maze compared. *Cognitive Brain Research, 3*, 167-181.

Hodges, H., Veizovic, T., Bray, N., French, S. J., Rashid, T. P., Chadwick, A., & Patel, S. (2000). Conditionally immortal neuroepithelial stem cell grafts reverse age-associated memory impairments in rats. *Neuroscience, 101*, 945-955.

Hotchkiss, A. J., & Gibb, J. W. (1980). The long-term effets of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *Journal of Pharmacology and Experimental Therapeutics, 214*, 27-262.

Hynd, G. W., Hern, K. L., Novey, E. S., Eliopulous, D., Marshall, R., Gonzalez, J. J., & Voeller, K. K. (1993). Attention deficit hyperactivity disorder asymmetry of the caudate nucleus. *Journal of Child Neurology, 8*, 339-347.

Jasmine, L., & Ohara, P. T. (2005). CNS noradrenaline and pain. Retrieved February 10, 2005, from http://anatomy.ucsf.edu/ohara/noradrenaline_pain.pdf

Jung, A. B., & Bennett, J. P. (1996). Development of striatal dopaminergic function. I. Pre- and postnatal development of mRNAs and binding sites for striatal D1 (D1a) and D2 (D2a) receptors. *Brain Research Developmental Brain Research, 94*(2), 109-120.

Kelly, J. P. (1993). Neural basis of perception and movement. In E. R. Kandel, J. H. Schwartz, & T. M. Jessell (Eds.), *Principles of neural behavior* (4th ed., pp. 283-295). New York: Elsevier.

Kesslak, J. P., So, V., Choi, J., Cotman, C. W., & Gomez-Pinilla, F. (1998). Learning upregulates brain-derived neurotropic factor messenger ribonucleic acid: A mechanism to facilitate encoding and circuit maintenance? *Behavioral Neuroscience, 112*(4), 1012-1019.

Klove, H. (1989). The hyperarousal hypothesis: What is the evidence? In T. Sagvolden, & T. Archer (Eds), *Attention deficit disorder: Clinical and basic research* (pp. 131-136). Hillsdale, NJ: Lawrence Erlbaum Associates.

Kolta, M. G., Scalzo, F. M., Ali, S. F., & Holson, R. R. (1990). Ontogeny of the enhanced behavioral response to amphetamine in amphetamine-pretreated rats. *Psychopharmacology, 100,* 377-382.

Krivanek, J., & McGaugh, J. L. (1969). Facilitating effects of pre and post trial amphetamine administration on discrimination learning in mice. *Agents Actions, 1,* 36-42.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinepherine: Comparison with amphetamine. *Journal of Neurochemistry, 68,* 2032-2037.

Lauder, J. M., & Bloom, F. E. (1974). Ontogeny of monoamine neurons in the locus coeruleus, Raphe nuclei and substantia nigra of the rat. 1. Cell differentiation. *The Journal of Comparative Neurology, 155,* 469-468.

Leeds, P., Leng, Y., Chalecka-Franaszek, E., & Chuang, D. M. (2005). Neurotrophins protect against cytosine arabinoside-induced apoptosis of immature rat cerebellar neurons. *Neurochemistry International, 46,* 61-72.

Leith, N. J., & Kuczenski, R. (1981). Chronic amphetamine: Tolerance and reverse tolerence reflect different behavioral actions of the drug. *Pharmacology, Biochemistry and Behavior, 15,* 399-404.

Leith, N. J., & Kuczenski, R. (1982). Two dissociable components of behavioral sensitization following repeated amphetamine administration. *Psychopharmacology, 76,* 310-315.

Levine, E. S., Crozier, R. A., Black, I. B., & Plummer, M. R. (1998). Brain-derived neurotropic factor modulates hippocampal synaptic transmission by increasing N-methyl-D asparic acid receptor activity. *Proceeds National Academy of Science, 95,* 10235-10239.

Levy, F., & Hobbes, G. (1996). Does haloperidol block methylphenidate? Motivation or attention? *Psychopharmacology (Berl), 126,* 70-74.

Li, Y., Kolb, B., & Robinson, T. E. (2003). The location of persistent amphetamine-induced changes in the density of dendritic spines or medium spiny neurons in the nucleus accumbens and caudate-putamen. *Neuropsychology, 28*(6), 1082-1085.

Lucot, J. B., Wagner, G. C., Schuster, C. R., & Seiden, L. S. (1982). Decreased sensitivity of rat pups to long-lasting dopamine and serotonin depletions produced by methylamphetamine. *Brain Research, 247*, 181-183.

Markowitz, J. S., & Patrick, K. S. (2001). Pharmacokinetic and pharmacodynamic drug interactions in the treatment of attention-deficit hyperactivity disorder. *Clinical Pharmacokinetics, 40*, 753-772.

Mason, S. T. (1984). *Catecholamines and behaviour.* New York: Cambridge University Press.

Matsuzaki, H., Namikawa, K., Kiyama, H., Mori, N., & Sato, K. (2004). Brain derived neurotrophic factor rescues neuronal death induced by methamphetamine. *Biological Phsychiatry, 55*, 52-60.

McDougall, S. A., Duke, M. A., Bolanos, C. A., & Crawford, C. A. (1994). Ontogeny of behavioral sensitization in the rat: Effects of direct and indirect dopamine agonists. *Psychopharmacology (Berl), 116*, 483-490.

Meredith, G. E., Callen, S., & Scheuler, D. A. (2002). Brain-derived neurotropic factor expression is increased in the rat amygdala, piriform cortex, and hypothalamus following repeated amphetamine administration. *Brain Research, 949,* 218-227.

Micheau, J., & Riedel, G. (1999). Protien kinases: Which one is the memory molecule? *Cellular and Molecular Life Science, 55,* 534-48.

Micheli, M. R., Bova, R., Laurenzi, M. A., Bazzucchi, M. & Grassi Zucconi, G. (2006). Modulation of BDNF and TrkB expression in rat hippocampus in response to acute neurotoxicity by diethyldithiocarbamate. *Neuroscience Letters, 410,* 66-10.

Miele, M., Mura, M.A., Enrico, P., Esposito, G., Serra, P. A., Migheli, R., Zangani, D., Miele, E., & Desole, M. S. (2000). On the mechanisms of d-amphetamine-induced changes in glutamate, ascorbic acid and uric acid release in the striatum of freely moving rats. *British Journal of Pharmacology, 129,* 582-588.

Milner, B., Squire, L. R., & Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron, 20,* 445-468.

Mizuno, M., Yamada, K., Maekawa, N., Kuniaki, S., Seishima, M., & Nabeshima, T. (2002). CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. *Behavioural Brain Research, 113,* 135-141.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., & Nabeshima, T. (2000). Involvement of brain-derived neurotropic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *The Journal of Neuroscience, 20,* 7116-7121.

Morris, M. J., Dausse, J. P., Devynck, M. A., Myer, P. (1980). Ontogeny of α1 and α2 adrenoreceptors in rat brain. *Brain Research, 190,* 268-271.

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Memory, 12,* 239-260.

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

Mu, J. S., Li, W. P., Yao, Z. B., & Zhou, X. F. (1999). Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Research, 835,* 259-265.

National Institute of Health Consensus Development Statement. (1998). Diagnosis and treatment of attention deficit hyperactivity disorder. *National Institute of Health Consensus Statement, Nov 16-18;* 16, 1-37.

Needleman, H. L., Gunnoe, C., Leviton, A., Reed, R., Peresie, H., Maher, C., & Barrett, P. (1979). Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *New England Journal of Medicine, 331,* 616-617.

Nestler, E. J. & Aghahanian, G. K. (1997). Molecular and cellular basis of addiction. *Science, 278,* 58-63.

Nomura, Y., Yotsumoto, I., & Segawa T. (1981). Ontogenic development of high potassium and acetylcholine induced release of dopamine from striatal slices of the rat. *Developmental Brain Research, 1,* 171-177.

Obernier, J. A., White, A. M., Swartzwelder, H. S., & Crews, F. T. (2002). Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. *Pharmacology, Biochemistry, and Behavior, 72,* 521-532.

Olton, D. S., & Samuelson, R. J. (1976). Remembrance of places past: Spatial memory in rats. *Journal of Experimental Psycholgy: Animal Behavior Processes, 2,* 97-116.

Ou, L. C., & Gean, P. W. (2007). Transcriptional regulation of brain-derived neurotrophic factor in the amygdala during the consolidation of fear memory. *Molecular Pharmacology, 72,* 350-358.

Paule, M. G., Rowland, A. S., Ferguson, S. A., Chelonis, J. J., Tannock, R., Swanson, J. M., & Castellanos, F. X. (2000). Attention deficit/hyperactivity disorder: Characteristics, interventions, and models. *Neurotoxicology and Teratology, 2,* 631-651.

Pelham, W. E. Jr., Wheeler, T., & Chronis, A. (1998). Empirically supported psychosocial treatments for attention deficit hyperactivity disorder. *Journal of Clinical Child Psychology, 27,* 190-205.

Perrot-Sinal, T. S., Kostenuik, M. A., Ossenkopp, K. P., & Kavaliers, M. (1996). Sex differences in performance in the Morris water maze and the effects of initial nonstationary hidden platform training. *Behavioral Neuroscience, 110,* 1309-1320.

Pioli, E. Y., Meissner, W., Sohr, R., Gross, C. E., Bezard, E., & Bioulac, B. H. (2008). Differential behavioral effects of partial bilateral lesions of ventral tegmental area or substantia nigra pars compacta in rats. *Neuroscience, 153*, 1213-1224.

Pitts, D. K., Freeman, A. S., & Chiodo, L. A. (1990). Dopamine neuron ontogeny: Electrophysiolgical studies. *Synapse, 6*, 309-320.

Porrino, L. J., Lucignani, G., Dow-Edwards, D., & Sokoloff, L. (1984). Correlation of dose-dependent effects of acute amphetamine administration on behavior and local cerebral metabolism in rats. *Brain Research, 307*, 11-320.

Pu, C., & Vorhees, C. V. (1993). Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in rat striatum. *Developmental Brain Research*, 72, 325-328.

Reitan, R. M., & Wolfson, D. (1985). *Neuroanatomy and neuropathology: A clinical guide for neuropsychologist*. Tuscon, AZ: Neuropsychology Press.

Renner, M. J., & Seltzer, C. P. (1991). Molar characteristics of exploratory and investigative behavior in the rat (*Rattas noregicus*). *Journal of Comparative Psychology, 105*, 326-339.

Rho, J. M., & Storey, T. W. (2001). Molecular ontogeny of major neurotransmitter receptor systems in the mammalian central nervous system: Norepinephrine, dopamine, serotonin, acetylcholine, and glycine. *Journal of Child Neurology, 16,* 271-280.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methamphetamine in the rat brain. *Brain Research, 235,* 93-103.

Ricaurte, G. A., Schuster, C. R., & Sieden, L. S. (1980). Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Research, 193,* 153-163.

Ricaurte, G. A., Seiden, L. S., & Shuster, C. R. (1984). Further evidence that amphetamine produces long-lasting dopamine neurochemical deficits by destroying dopamine nerve fibers. *Brain Research, 303,* 359-364.

Robinson, L. M., Sclar, D. A., Skaer, T. L., & Galin, R. S. (2008). Treatment modalities among U.S. children diagnosed with attention-deficit hyperactivity disorder: 1995-99. The Annals of Pharmacotherapy, *42,* 24-31.

Robinson, T. E., & Kolb, B. (1997). Persistent structural modifications in the nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. Journal of Neuroscience, *17*(21), 8491-8797.

Roof, R. L., & Stein, D. G. (1999). Gender differences in Morris water maze performance depend on task parameters. *Physiology and Behavior, 68,* 81-86.

Rossetti, Z. L., & Carboni, S. (2005). Noradrenaline and dopamine elevations in the rat prefrontal cortex in spatial working memory. *Journal of Neuroscience, 25,* 2322-2329.

Roth, R. H., & Nowycky, M. C. (1977). Non striatal dopaminergic neurons: Role of presynaptic receptors in the modulation of transmitter synthesis. *Advances in Biochemical Psychopharmacology,* 77, 465-470.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C., & Taljaard, J. J. (1998). Differences between electrically-ritalin, and d-amphetamine-stimulated release of [^{3}H]dopamine from brain slices suggest impaired vesicular storage of dopamine in an animal model of attention deficit hyperactivity disorder. *Behavioral Brain Research, 94,* 163-171.

Russig, H., Durrer, A., Yee, B. K., Murphy, C. A., & Feldon, J. (2003). The acquisition, retention and reversal of spatial learning in the Morris water maze task following withdrawal from an escalating dosage schedule of amphetamine in Winstar rats. *Neuroscience, 119,* 167-179.

Sagvolden, T., & Archer, T. (1989). *Attention deficit disorder; Clinical and basic research.* Hillsdale, NJ: Lawrence Erlbaum Associates.

Sagvolden, T., & Seargeant, J. A. (1998). Attention deficit/hyperactivity disorder-from brain dysfunction to behavior. *Behavioral Brain Research, 130,* 191-196.

Sandstrom, N. J., & Rowan, M. H. (2007). Acute pretreatment with estradiol protects against CA1 cell loss and spatial learning impairments in the Morris water maze. *Hormones and Behavior, 51,* 335-345.

Santana, C., Rodriguez, M., Alfonso, D., & Arevalo, R. (1992). Dopaminergic neuron development in rats: Biochemical study from prenatal life to adulthood. *Brain Research Bulletin, 29,* 7-13.

Scharchar, R. J., Tannock, R., & Logan, G. (1993). Inhibitory control, impulsiveness, and attention deficit hyperactivity disorder. *Clinical Psychology Review, 13,* 721-739.

Schroder, N., O'Dell, S. J., & Marshall, (2003). Neurotoxic mehtamphetamine regimen severely impairs recognition memory in rats. *Synapse, 49,* 89-96.

Sharp, T., Zetterstrom, T., Ljungberg, T., & Ungerstedt, U. (1987). A direct comparison of amphetamine induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. *Brain Research, 401,* 322-330.

Shobe, J. (2001). The role of PKA, CaMKII, and PKC in avoidance conditioning: Permissive of instructive? *Neurobiology of Learning & Memory,* 77, 291-312.

Shore, P. A., & Dorris, R. L. (1975). On a prime role for newly synthesized dopamine in striatal function. *European Journal of Pharmacology, 30,* 315-318.

Sibley, D. R., & Monsma, F. J. Jr. (1992). Molecular biology of dopamine receptors. In G. J. Siegel, B. A. Agranoff, R. W. Albers, S. K. Fisher & M. D. Uhler (Eds.), Basic Neurochemistry: Molecular, cellular, and medical aspects (pp. 254). Philadelphia: Lippincott-Raven.

Sieden, L. S., & Shuster, C. R. (1985). Persistent effects of amphetamine, p-chloroamphetamine, and related compounds on central dopamine and serotonin neurons in rodents. *Psychopharmacology Bulletin, 21,* 528-532.

Singh, M., Meyer, E. M., & Simpkins J.W. (1995). The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology, 135,* 2320-2324.

Smidts, D. P., Oosterlaan, J. (2007). How common are symptoms of ADHD in typically developing preschoolers? A study on prevalence rates and prenatal/demographic risk factors. *Cortex, 43,* 710-717.

Spencer, T. J., Beiderman, J., Harding, M., O'donnell, D., Farone, S. V. & Willens, T. E. (1996). Growth deficits in ADHD children revisited: Evidence for disorder-associated growth delays? *Journal of the American Academy of Child and Adolescent Psychiatry, 35,* 1460-1469.

Srivasta, L. K., Morency, M. A., & Mishra, R. K. (1992). Ontogeny of dopamine D2 receptor mRNA in rat brain. *European Journal of Pharmacology, 225,* 143-150.

Sugiyama, N., Kanba, S., & Arita, J. (2003). Temporal changes in the expression of brain-derived neurotrophic factor mRNA in the ventromedial nucleus of the hypothalamus of the developing rat brain. *Brain Research. Molecular Brain Research, 115,* 69-77.

Sulzer, D., Maidment, N. T., & Rayport, S. (1993). Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain structures. *Journal of Neurochemistry, 60,* 527-535.

Swanson, J., Flodman, P., Kennedy, J., Spence M. A., Moyzis, R., Schuck S., Murias, M., Moriarty, J., Barr, C., Smith, M., & Posner, M. (2000). Dopamine genes and ADHD. *Neuroscience Biobehavioral Review, 24,* 21-25.

Tarazi, F. I., Tomasini, E. C., & Baldessarini, R. J. (1998). Postnatal development of dopamine D4-like receptors in rat forebrain regions: Comparison with D2-like receptors. *Brain Research Developmental Brain Research, 110,* 227-33.

U'Prichard, D. C., & Snyder, S. H. (1979). Distinct α-noradrenergic receptor differentiated by binding and physiological relationships. In G. J. Siegel, B. A. Agranoff, R. W. Albers, S. K. Fisher & M. D. Uhler (Eds.), *Basic Neurochemistry: Molecular, cellular, and medical aspects* (pp. 258). Philadelphia: Lippincott-Raven.

Ulloa, R., Nicolini, H., & Fernandez-Guasti, A. (2004). Age differences in an animal model of obsessive-compulsive disorder: participation of dopamine. Dopamine in an animal model of OCD. *Pharmacology, Biochemistry and Behavior, 78,* 661-666.

Van der Zee, C. E., Lourenssen, S., Stanisz, J., & Diamond, J. (1995). NGF deprivation of adult rat brain results in cholinergic hypofunction, and selective impairments in spatial learning. *European Journal of Neuroscience,* 7, 160-168.

Varley, C. K. (1984). Attention deficit disorder (the hyperactivity syndrome): A review of selected issues. *Developmental and Behavioral Pediatrics,* 5, 254-258.

Vorhees, C. V., Inman-Wood, S. L., Morford, L.L., Broeing, H. W., Fukumura, M., & Moran, M. S., (2000). Adult learning deficits after neonatal exposure to d-methamphetamine: Selective effects on spatial navigation and memory. *Journal of Neuroscience, 20,* 4732-4739.

Vorhees, C. V., Skelton, M. R., & Williams, M. T. (2007). Age-dependent effects of neonatal methamphetamine exposure on spatial learning. *Behavioral Pharmacology, 18,* 549-562.

Wagner, G. C., Ricaurte, G. A., Seiden, L. S., Schuster, C. R., Miller, R. J., & Westley, J. (1980). Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Research, 181,* 151-160.

Wagner, G. C., Schuster, C. R., & Seiden, L. S. (1981). Neurochemical consequences following administration of CNS stimulants to the neonatal rat. *Pharmacology Biochemistry and Behavior, 14,* 117-119.

Walker, R. J. (1986). Biosynthesis, storage, and release of dopamine (pp. 3-25). In W. Winlow and R. Markenstien (Eds) *The neurobiology of dopamine systems.* Dover, NH: Manchester University Press.

Wargin, W., Kilts, P. C., Gualtieri, C. T., Ellington, K. R., Mueller, R. A., Kraemer, G., & Breese, G. R. (1983). Pharmacokinetics of methylphenidate in man, rat and monkey. *Journal of Pharmacology and Experimental Therapeutics, 226,* 382-386.

Waslick, B., & Greenhill, L. (1997). Attention deficit hyperactivity disorder. In J. M. Weiner (Ed) *Textbook of child and adolescent psychiatry* (2nd ed., pp. 389-410) Washington D.C.: American Academy of Child and Adolescent Psychiatry, American Psychiatric Press.

Whishaw, I. Q. (1998). Place learning in hippocampal rats and the path integration hypothesis. *Neuroscience and Biobehavioral Reviews, 22,* 209-220.

Williams, M. T., Morford, L. L., Wood, S. L., Wallace, T. L., Fukumura, M., Broening, H. W., & Vorhees, C. V. (2003). Developmental D-methamphetamine treatment selectively induces spatial navigation impairments in reference memory in the Morris water maze while sparing working memory. *Synapse, 48,* 138-148.

Wood, E. R., Dudchenko, P. A., & Eichenbaum, H. (1999). The global record of memory in hippocampal neuronal activity. *Nature, 397,* 561-563.

Yao, W. D., & Wu, C. F. (2001). Distinct roles of CaMKII and PKA in regulation of firing patterns and K(+) currents in Drosophilia neurons. *Journal of Neurophysiology, 85,* 1384-1394.

Yotsumoto, I., & Nomura, Y. (1981). Ontogenesis of the dopamine uptake into P2 fractions and slices of the rat brain. *Japanese Journal of Pharmacology, 31,* 298-300.

Zametikin, A. J., Nordahl, T. E., Gross, M., King, A. c., Semple, W. E., Rumsey, J., Hamburger, S., & Cohen, R. M. (1990). Cerebral glucose metabolism in adults with hyperactivity of childhood onset. *New England Journal of Medicine, 323,* 1361-1366.

Zhou, J., Zhang, H., Cohen, R. S., & Pandey, S. C. (2005). Effects of estrogen treatment on expression of brain-derived neurotrophic factor and camp response element-binding protein expression and phosphoryation in rat amygdaloid and hippocampal structures. *Neuroendocrinology, 81,* 294-310.

Zito, J. M., Safer, D. J., dos Reis, S., Gardner, J. F., Boles, M., & Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. *Journal of the American Medical Association, 283,* 1025-1030.