2015

Understanding comorbid ADHD and cocaine abuse: consequences of adolescent medication in an animal model

BOSTON UNIVERSITY

GRADUATE SCHOOL OF ARTS AND SCIENCES

Dissertation

UNDERSTANDING COMORBID ADHD AND COCAINE ABUSE:

CONSEQUENCES OF ADOLESCENT MEDICATION IN AN ANIMAL MODEL

by

CHLOE JENNIFER JORDAN

B.A., Boston University, 2010
M.A., Boston University, 2011

Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

2015

Approved by

First Reader

_____

Kathleen M. Kantak, Ph.D.
Professor of Psychological and Brain Sciences

Second Reader

_____

Linda P. Dwoskin, Ph.D.
Associate Dean for Research
Endowed Professor in Pharmaceutical Education
University of Kentucky, College of Pharmacy

Third Reader

_____

Gary B. Kaplan, M.D.
Professor of Psychiatry, Pharmacology and Experimental Therapeutics
Boston University School of Medicine

ACKNOWLEDGEMENTS

Completing this dissertation has been a journey, and there are a number of special people who have helped me along the path. My deepest gratitude goes towards Dr. Kathleen Kantak, for all of her mentorship and support during my graduate years. With unyielding patience (and many track changes) Kat has taught me how write, how to give talks, how to analyze data and navigate the idiosyncrasies of graphing in SigmaPlot. With her magic hands, Kat has shown me how to fix ghost-like equipment malfunctions, how to troubleshoot programs, how to deal with criticism and rejection, and then move on and try again. Kat has supported me in teaching and managing personalities of all types both in the lab and beyond, and has given me an appreciation of the value of car rides for coming up with new ideas. These are but a small few of the things I have learned from Kat, which I will carry with me throughout my career. I am eternally grateful to her.

I would also like to thank the members of my committee. Dr. Linda Dwoskin, for her thoughtful recommendations and insights on many papers and abstracts, and for challenging me to think critically ever since our first phone conference. Dr. Gary Kaplan, for his valuable input and time, even in the early stages of my prospectus. Dr. James Cherry, both for being a scientific resource and for showing me how to design a class, a skill that I will implement one day teaching courses of my own. I owe a special thank you to Dr. Helen Tager-Flusberg, who helped me publish my very first paper. My early years in Helen's lab played an integral role in my decision to pursue a research career. Helen has continued to be a wonderful, supportive influence in classes and beyond ever since.

There are a number of other individuals who I am especially grateful towards, for their essential roles in my scientific and personal development. Dr. Jacqueline Liederman, for showing me how to give captivating lectures even during one of the worst winters Boston has ever seen. Dr. Michael Baum, for the coffee and his valuable advice on navigating the next stages of my career. I am especially grateful to Dr. Catherine Caldwell-Harris, for her for inspiring me with her creativity, for guiding me and giving me the freedom to flourish in my undergraduate years, and for her unwavering positive reinforcement that kindled my passion for research in the first place.

I also would like to thank the many stellar students with whom I have had the fortune to work with over the years. These individuals not only contributed to the success of the experiments in this dissertation, but also were the lights in each day I spent in the lab. In particular, I tremendously grateful to Britahny Baskin, who came into the lab at just the right time and who has grown to become one of my most valued and trusted friends. I would also like to thank Danielle Taylor, for her inspiring stories and for coaching even the slowest SHR through the set shift. Carley Lemay, for her tireless energy, Laura Tabbaa, for reminding me how exciting science can be. Katherine Rodriguez, for her creativity, Malak El-Quessny, for her hands. Sae-Mi Jeon, Angelica Dellamorte, and Marcela Cruz, for always being awesome. And my lab mates: Jamie Gauthier, for commiserating, Jono, Roxann, and Bríd, who were there at the beginning.

Finally, I am tremendously grateful to my old friends, and especially my mom, my dad, and my partner in love and life, Dane. You have kept me grounded and sane, reminded me to enjoy the ride, and always helped me keep the big picture in sight.

UNDERSTANDING COMORBID ADHD AND COCAINE ABUSE: CONSEQUENCES OF ADOLESCENT MEDICATION IN AN ANIMAL MODEL

(Order No.)

CHLOE JENNIFER JORDAN

Boston University Graduate School of Arts and Sciences, 2015

Major Professor: Kathleen M. Kantak, Ph.D., Professor of Psychological and Brain Sciences

ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is highly comorbid with substance use disorders, particularly cocaine. Preclinical studies using the well-validated Spontaneously Hypertensive Rat (SHR) model of ADHD suggest that adolescent treatment with the stimulant methylphenidate increases cocaine abuse risk in adulthood, highlighting the need to identify alternative medications for teenagers with ADHD. Experiments 1-4 tested the hypothesis that atomoxetine, a non-stimulant that improves prefrontal cortex functioning in adolescent SHR, would not increase cocaine abuse risk. The speed to acquire cocaine self-administration, the efficacy and motivating influence of cocaine reinforcement, and reactivity to cocaine cues in adulthood following discontinuation of adolescent atomoxetine treatment were examined in male SHR and two genetic control strains: inbred Wistar-Kyoto (WKY) and outbred Wistar (WIS). Because atomoxetine is not always as clinically efficacious as methylphenidate, Experiments 5-9 tested the hypothesis that an alternative stimulant, d-amphetamine, would improve cognitive performance in adolescent SHR during a strategy set-shifting

task and not increase cocaine abuse risk in adult SHR after adolescent d-amphetamine was discontinued. Across experiments, adult SHR acquired cocaine self-administration faster than control strains and also were more sensitive to cocaine's reinforcing and motivating influence and more reactive to cocaine cues. As hypothesized, adolescent atomoxetine did not increase any measure of cocaine abuse risk in adult SHR and modestly reduced SHR's reactivity to cocaine cues. In WKY control, however, adolescent atomoxetine accelerated acquisition of cocaine self-administration. d-Amphetamine improved set-shifting deficits in adolescent SHR, demonstrating pro-cognitive effects as hypothesized. When self-administration was acquired, cocaine intake was lower in adult SHR that received adolescent d-amphetamine compared to vehicle-treated SHR, consistent with the hypothesis. Adolescent d-amphetamine slowed acquisition and reduced the efficacy and motivating influence of cocaine reinforcement in WIS control, but accelerated acquisition in WKY control. Collectively, these results highlight the heuristic value of SHR in evaluating comorbid ADHD and cocaine abuse risk, and suggest that atomoxetine and d-amphetamine may be safer medications than methylphenidate for teenagers with ADHD. However, findings in control strains emphasize the need for accurate ADHD diagnosis, as the long-term consequences of treatment could be favorable (d-amphetamine in WIS) or unfavorable (atomoxetine and d-amphetamine in WKY) in misdiagnosed individuals.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS....iv

ABSTRACT....vi

LIST OF TABLES....xii

LIST OF FIGURES....xiii

LIST OF ABBREVIATIONS....xv

CHAPTER ONE....1

General Introduction....1

ADHD and Cocaine Abuse....2

Stimulant Drugs and Sensitization....4

Changes in Cocaine Abuse Risk following Treatment for ADHD....6

Adolescence: A Sensitive Period....9

The Spontaneously Hypertensive Rat Model of ADHD....11

CHAPTER TWO....16

Aim 1: Treatment with a Non-Stimulant ADHD Medication During Adolescence and Cocaine Abuse Risk and Cocaine Cue Reactivity in Adulthood....16

Introduction....16

Experiment 1: Acquisition of Cocaine Self-Administration Following Adolescent Atomoxetine Treatment....21

Materials and Methods 22
Results 28
Experiment 2: Fixed-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent Atomoxetine Treatment 31
Materials and Methods 32
Results 33
Experiment 3: Progressive-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent Atomoxetine Treatment 35
Materials and Methods 36
Results 37
Experiment 4: Cocaine Cue Reactivity and Relapse Behavior in Adulthood Following Adolescent Atomoxetine Treatment 41
Materials and Methods 41
Results 44
Discussion 50
Strain Differences in Cocaine Abuse Risk and Cocaine Cue Reactivity 50
Effects of Adolescent Atomoxetine on Cocaine Abuse Risk and Cue Reactivity 53
CHAPTER THREE. 56
Aim 2: Effects of an Alternative Stimulant Medication on Prefrontal Cortex Function during Adolescence and Cocaine Abuse Risk and Cue Reactivity in Adulthood 56
Introduction 56

Experiment 5: Effects of d-Amphetamine on Strategy Set Shifting in Adolescent Rats 61
Materials and Methods 61
Results 68
Experiment 6: Acquisition of Cocaine Self-Administration Following Adolescent d-Amphetamine Treatment 75
Materials and Methods 75
Results 76
Experiment 7: Fixed-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent d-Amphetamine Treatment 79
Materials and Methods 80
Results 80
Experiment 8: Progressive-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent d-Amphetamine Treatment 83
Materials and Methods 83
Results 84
Experiment 9: Cocaine Cue Reactivity and Relapse Behavior in Adulthood Following Adolescent d-Amphetamine Treatment 88
Materials and Methods 88
Results 90
Discussion 97
Strain Differences in Prefrontal Cortex Functioning in Adolescence 97

Effects of d-Amphetamine Treatment on Prefrontal Cortex Functioning in Adolescence 102

Strain Differences in Cocaine Abuse Risk and Cocaine Cue Reactivity in Adulthood 103

Effects of Adolescent d-Amphetamine Treatment on Cocaine Abuse Risk and Cue Reactivity in Adulthood 106

CHAPTER FOUR. 108

General Discussion 108

Importance of the Spontaneously Hypertensive Rat Model of ADHD 108

Significance of Adolescent Atomoxetine Treatment on Cocaine Abuse Risk in Adulthood 110

Significance of Adolescent d-Amphetamine Treatment on Cocaine Abuse Risk in Adulthood 115

Treatment and Prevention of Cocaine Abuse 121

Future Directions 125

Limitations 126

Conclusions 130

BIBLIOGRAPHY 132

CURRICULUM VITAE 159

LIST OF TABLES

Table 1. Effects of ADHD Medications on Prefrontal Cortex Function in Adolescence and Cocaine Abuse Risk in Adulthood 123

Table 2. Monoamine Transporter Functioning in Prefrontal Cortex of Untreated and Treated Rats 124

Table 3. Proposed Role of Monoamine Transporters in Behavior 125

LIST OF FIGURES

Figure 1 23

Figure 2 29

Figure 3 30

Figure 4 31

Figure 5 34

Figure 6 35

Figure 7 38

Figure 8 39

Figure 9 40

Figure 10 45

Figure 11 46

Figure 12 46

Figure 13 47

Figure 14 48

Figure 15 49

Figure 16 50

Figure 17 62

Figure 18 65

Figure 19 65

Figure 20 66
Figure 21 70
Figure 22 72
Figure 23 74
Figure 24 77
Figure 25 78
Figure 26 79
Figure 27 81
Figure 28 82
Figure 29 85
Figure 30 86
Figure 31 87
Figure 32 91
Figure 33 92
Figure 34 92
Figure 35 93
Figure 36 94
Figure 37 95
Figure 38 96

LIST OF ABBREVIATIONS

ADHD ..... Attention-Deficit/Hyperactivity Disorder

AMPA ..... α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPH ..... d-Amphetamine

ANOVA ..... Analysis of Variance

ATO ..... Atomoxetine

BDNF ..... Brain Derived Neurotrophic Factor

Cr ..... Charles River

CREB ..... Cyclic Adenosine Monophosphate Response-Element Binding Protein

DAT ..... Dopamine Transporter

DAT-1 ..... Dopamine Transporter-1 Gene

DLPFC ..... Dorsolateral Prefrontal Cortex

ERK ..... Extracellular Signal-Related Kinases

Ext ..... Extinction

kg ..... Kilogram

FI ..... Fixed Interval

FR ..... Fixed Ratio

hr ..... Hour(s)

i.d. ..... Inner Diameter

IFG ..... Inferior Frontal Gyrus

i.p. ..... Intraperitoneal

IU ..... International Units

i.v. ..... Intravenous

mg ..... Milligrams

ml ..... Milliliters

min ..... Minutes

mm ..... Millimeters

mPFC ..... Medial Prefrontal Cortex

mRNA ..... Messenger Ribonucleic Acid

MTA ..... Multimodal Treatment of Attention Deficit Hyperactivity Disorder Study

NBER ..... National Bureau of Economic Research

NIDA ..... National Institute of Drug Abuse

NMDA ..... N-Methyl-D-aspartate

NSCH ..... National Survey of Children's Health

NET ..... Norepinephrine Transporter

o.d. ..... Outer Diameter

OFC ..... Orbitofrontal Cortex

P(#) ..... Postnatal Day #

PFC ..... Prefrontal Cortex

PR ..... Progressive Ratio

SAMHSA ..... Substance Abuse and Mental Health Services Administration

s.c. ..... Subcutaneous

sec ..... Second

SEM ..... Standard Error of the Mean

SERT ..... Serotonin Transporter

SHR ..... Spontaneously Hypertensive Rat

SUD ..... Substance Use Disorder

TAAR1 ..... Trace Amine Associated Receptor-1

VEH ..... Vehicle

VLPFC ..... Ventrolateral Prefrontal Cortex

VMAT-2 ..... Vesicular Monoamine Transporter-2

VTA ..... Ventral Tegmental Area

WIS ..... Wistar

WKY ..... Wistar-Kyoto

CHAPTER ONE.

General Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a widely prevalent neurodevelopmental disorder that affects 8-12% of children and adults worldwide, up to ~78% of whom may experience symptoms that persist in adulthood (Biederman et al, 2010; Visser et al, 2014). In the United States, ADHD diagnoses increased by 42% from 2003 to 2011, with rates escalating fastest among teenage boys aged 14 to 17 (Schwarz & Cohen, 2013; Visser et al, 2014). Adolescents and adults with ADHD are at a high risk of drug abuse. Comorbidity estimates of ADHD and substance use disorder (SUD) range from 9.5% up to 58%, depending on the sample (Sizoo et al, 2010; Steinhausen & Bisgaard, 2014). Moreover, ADHD is a significant predictor of SUD (Wilens et al, 2011), and is associated with earlier onset and increased severity of SUD, as well as greater functional impairments resulting from drug use (de los Cobos et al, 2011; Kousha et al, 2012). Although nicotine, alcohol, and cannabis represent the most frequently abused drugs by individuals with ADHD (Faraone et al, 2007), ADHD patients are also more likely than those without ADHD to escalate their use to include other illicit drugs, particularly cocaine (Biederman et al, 2006). The lifetime prevalence of cocaine use disorder in individuals with ADHD is 23%, more than double the prevalence of cocaine use disorder observed in the general population, which is 10.8% (Lambert, 2005). Consistent with these rates, meta-analysis has shown that children with ADHD are twice

as likely to abuse cocaine or to become cocaine dependent in adulthood, compared to children without ADHD (Lee et al, 2011). ADHD and cocaine abuse share several behavioral and neurological features, which may contribute to high comorbidity of these disorders.

ADHD and Cocaine Abuse

The core characteristics of ADHD include hyperactivity, impulsivity, and inattention (American Psychiatric Association, 2013). Other robust features of ADHD include deficits in inhibitory control, working memory, and behavioral flexibility, as well as elevated sensitivity to novelty (Barkley, 1997; Rapport et al, 2008; Tengelbeckers et al, 2015). Drug dependence is defined by a loss of control over drug consumption and continued drug use despite negative consequences (American Psychiatric Association, 2013). Consistent with this definition, cocaine-abusing individuals exhibit impulsivity and deficits in sustained attention, working memory, and inhibitory control (Burgess et al, 2010; Colzato et al, 2009; Hester & Garavan, 2004; Potvin et al, 2014; Vonmoos et al, 2013). However, it remains unclear whether these deficits contribute to the development of cocaine dependence, or whether they are a result of chronic drug use.

Several overlapping neural substrates underlie the behavioral features that ADHD and cocaine dependent individuals have in common. For example, during inhibitory control tasks, individuals with ADHD show reduced activation in the right inferior frontal gyrus (IFG), as well as in medial prefrontal and orbitofrontal cortices (mPFC and OFC, respectively), compared to individuals without ADHD (Booth et al, 2005; Cubillo et al,

2011; Depue et al, 2010; Rubia et al, 2005). Cocaine users similarly fail to show activation in mPFC or dorsolateral prefrontal cortex (DLPFC) during inhibitory control tasks, which is typically observed in non-drug using controls (Hester & Garavan, 2004). Moreover, during reward-based tasks, increased activation in the OFC is observed both in individuals with ADHD and in cocaine using subjects, compared to controls (Bolla et al, 2003; von Rhein et al, 2015).

Dysfunction in the dopamine neurotransmitter system may underlie many of the behavioral and neurobiological features that individuals with ADHD and cocaine dependent individuals have in common. For example, individuals with ADHD exhibit decreased dopamine D2 receptor availability (Volkow et al, 2010), and have specific polymorphisms in the dopamine transporter (DAT) -1 gene (Faraone et al, 2005; Fusar-Poli et al, 2012). These polymorphisms likely contribute to the abnormal DAT function and increased DAT levels observed in the striatum of ADHD patients (Fusar-Poli et al, 2012; Hesse et al, 2009). Notably, the striatum is a region in which the DAT-1 gene is predominantly expressed in the brain (Durston et al, 2005), and plays a critical role in motivation and reward-associated learning. Cocaine users similarly exhibit decreased D2 receptor availability and polymorphisms in the DAT-1 gene, in some of the same introns as those observed in ADHD (Asherson et al, 2007; Franke et al, 2010; Guindalini et al, 2006; Hoogman et al, 2012). Moreover, DAT-1 polymorphisms also likely contribute to abnormal DAT function in the striatum of cocaine users (Mash et al, 2002; Volkow et al, 2010). Interestingly, individuals with ADHD who receive stimulant medications for their symptoms exhibit increased DAT levels compared to medication-naïve ADHD patients

(Fusar-Poli et al, 2012), and cocaine abuse is associated with increased DAT function in the striatum (Mash et al, 2002). If long-term stimulant medication exacerbates DAT dysfunction in ADHD, this may also contribute to increased cocaine abuse risk in these individuals. The neural mechanisms associated with sensitization, an important consequence of repeated stimulant exposure, support this possibility.

Stimulant Drugs and Sensitization

Acutely, all drugs of abuse activate the mesocorticolimbic dopamine "reward" pathway, either directly or indirectly. The mesocorticolimbic pathway involves the ventral tegmental area (VTA), from which dopaminergic axons project to the nucleus accumbens and PFC. Drugs of abuse are thought to "hijack" this pathway by augmenting dopamine signaling (Buchta & Riegel, 2015). For example, cocaine inhibits monoamine transporters, serving to increase extracellular dopamine and other monoamines. Drug-seeking and drug-taking behaviors are thus reinforced in place of the pursuit of natural rewards, such as food, which do not increase extracellular dopamine to the same magnitude (Buchta & Riegel, 2015). Cocaine use also affects other brain regions, many of which have reciprocal connections with the nucleus accumbens and VTA. These include the striatum, amygdala, hippocampus, and cingulate cortex (Steketee & Kalivas, 2011), as well as specific subregions of the PFC, such as mPFC/DLPFC and OFC, as reviewed above (Bolla et al, 2003; Booth et al, 2005; Hester & Garavan, 2004).

Rather than causing tolerance, long-term or repeated administration of stimulant drugs may induce sensitization. Sensitization is broadly defined as enhanced

responsiveness to the drugs' effects, both with respect to behavior and within the brain. For example, it is well established in rodents that repeated administration of low doses of cocaine increases locomotor activity (see Steketee & Kalivas, 2011, for review). The reinforcing value of the drug, as well as the salience of drug-related cues, can also increase with repeated stimulant use (Robinson & Berridge, 1993). A number of neural changes are thought to underlie sensitization to drugs and drug-related cues. These include increases in dopamine and glutamate release in the VTA, nucleus accumbens, and mPFC, in response to stimulant administration (Steketee & Kalivas, 2011). In particular, increased activation of D1 receptors in these regions appears to be critical to the development of sensitization (Steketee & Kalivas, 2011). For example, D1 receptor responsivity in the nucleus accumbens increases with repeated cocaine administration (Henry & White, 1991). Moreover, infusion of D1 agonists into the nucleus accumbens or VTA augments sensitization to cocaine (De Vries et al, 1998; Pierce et al, 1996). In contrast, D1 antagonists in the VTA block the development of sensitization (Vezina, 1996). Similarly, antagonists of dopamine D1 and glutamate receptors in mPFC block the formation of sensitization to stimulant drugs (Li et al, 1999; Ramos et al, 2005).

Sensitization has also been linked to other synaptic changes in the mesocorticolimbic dopamine pathway. For example, one measure of excitatory synaptic strength is the ratio of AMPA to NMDA glutamate receptors (AMPAR/NMDAR). In the VTA, neurons showed elevated AMPAR/NMDAR ratios for up to 1 week following a single cocaine injection, and for up to 3 months following chronic cocaine self-administration (Chen et al, 2008; Lüscher & Malenka, 2011). Importantly, changes in

VTA plasticity following cocaine administration are dependent on D1 receptor activation (Schilström et al, 2006; Steketee & Kalivas, 2011). In contrast to the VTA, excitatory synaptic strength in the nucleus accumbens may be reduced by repeated cocaine administration. Decreased AMPAR/NMDAR ratios are observed in medium spiny neurons for up to 14 days after the last cocaine exposure (Lüscher & Malenka, 2011; Kourrich et al, 2007; Thomas et al, 2001).

One additional neurochemical change important to sensitization occurs at monoamine transporters. For example, DAT inhibition is necessary for cocaine-induced changes in nucleus accumbens plasticity, as well as cocaine-induced locomotor sensitization (Martin et al, 2011). Interestingly, continuous access to cocaine reduces cocaine's ability to block DAT (Calipari et al, 2013). In contrast, intermittent access to cocaine, which may be more representative of "real-world" cocaine use, increases cocaine's potency at DAT (Calipari et al, 2013). Importantly, increases in DAT are associated with ADHD, and may be a consequence of stimulant medication (Fusar-Poli et al, 2012; Hesse et al, 2009). Based on this understanding of sensitization, concern has been raised regarding the treatment of ADHD with stimulant drugs.

Changes in Cocaine Abuse Risk following Treatment for ADHD

In the United States, two-thirds of individuals diagnosed with ADHD receive a stimulant medication for their symptoms (Schwarz & Cohen, 2013). The most commonly prescribed stimulant medication is methylphenidate (Ritalin®; Castle et al, 2007; Raman et al, 2015; Visser et al, 2014). Methylphenidate is considered the first-line treatment for

ADHD (MTA Cooperative Group, 1999), and can result in up to 50% reduction in ADHD symptoms (Buitelaar et al, 2011; Scahill et al, 2004). Methylphenidate acts by inhibiting reuptake at DAT and at norepinephrine transporters (NET), resulting in increased extracellular concentrations of dopamine and norepinephrine (Gatley et al, 1996; Wilens, 2006). Regionally, in boys with ADHD methylphenidate increases right IFG activity, and normalizes hypoactivation in the ventrolateral PFC (VLPFC), which is associated with improved inhibitory control compared to placebo administration (Cubillo et al, 2012; Rubia et al, 2014). Increased stimulation at dopamine D1 and alpha2A noradrenergic receptors is thought to underlie methylphenidate's acute cognitive-enhancing effects (Arnsten & Dudley 2005; Gamo et al 2010).

Although methylphenidate may be efficacious in improving ADHD symptoms in the short-term, controversy remains regarding chronic treatment (Sibley et al, 2014). After moderate- and long-term follow up, increases in methylphenidate use were associated with increased school grade repetition and deteriorating parental relationships for boys with ADHD, and with unhappiness, depression, lower math scores, reduced higher education attendance, as well as deteriorating parental relationships for girls with ADHD (Currie et al, 2014; NBER, 2015). Further, as suggested above, controversy has risen regarding the relationship between methylphenidate treatment and increased risk of SUD in adulthood, beyond the risk conveyed by having ADHD alone. An early meta-analysis reported that stimulant treatment is protective against SUD (Wilens et al, 2003). However, the studies that were examined in the analysis failed to consider whether stimulant treatment was initiated in childhood vs. adolescence, or was ongoing vs.

discontinued at the time of assessment. Because substance use may be a form of self-medication for individuals with ADHD (Gudjonsson et al, 2012), ongoing methylphenidate treatment may compromise detection of increased cocaine abuse risk (Baskin et al, 2015; Schenk & Izenwasser, 2002). Moreover, clinical studies also often fail to distinguish between different types of stimulant medication, which may have different mechanisms of action and potentially different long-term behavioral effects, a possibility that is explored in Chapter Three. Finally, many studies in human subjects typically employ a limited follow-up period in adult participants. Because cocaine use generally develops later in life (Degenhardt et al, 2008), participants evaluated in their late teens and early twenties may not have surpassed the risk period for cocaine use (Humphreys et al, 2013).

Despite these limitations, current consensus is that initiation of stimulant treatment in childhood neither protects against, nor promotes, the risk of SUD (Humphreys et al, 2013; Molina et al, 2013; Volkow et al, 2008). However, controversy remains regarding initiation of stimulant medication during adolescence and the long-term consequences for SUD. When age of treatment onset is analyzed, evidence emerges indicating that the later in childhood that treatment is initiated, the greater the increase in the risk of developing SUD (Dalsgaard et al, 2014; Lambert et al, 1998; Manuzza et al, 2008; Steinhausen & Bisgaard, 2014). Several factors have been suggested to mediate this link between the age at which stimulant treatment is initiated and later substance abuse, including the number of active ADHD symptoms, the duration of untreated symptoms, and the development of antisocial personality disorder. However, the

cumulative duration of stimulant treatment is not associated with development of SUD (Dalsgaard et al, 2014), and conduct problems, which are precursors to antisocial personality disorder, do not differ between patients who initiate ADHD medication in childhood vs. adolescence (Mannuzza et al, 2008; Upadhyaya et al, 2005). These findings suggest that the duration of untreated symptoms and antisocial personality disorder cannot fully account for increased SUD following adolescent stimulant treatment. Importantly, adolescence is a distinct developmental period characterized by elevated plasticity in the mesocorticolimbic system. The unique neurological changes occurring during adolescence may therefore render the brain more susceptible to the sensitizing effects of stimulant drugs.

Adolescence: A Sensitive Period

Adolescence may be a vulnerable period when stimulants, which directly influence monoaminergic activity in the mesocorticolimbic system, have unique effects on reward responsivity (Andersen, 2005). Regionally, healthy teenagers exhibit hypoactivity in the some of the same brain regions that are dysfunctional in both individuals with ADHD and cocaine abusers, including the mPFC/DLPFC and OFC (Bolla et al, 2003; Booth et al, 2005; Hester & Garavan, 2004; Stanger et al, 2013). Interestingly, hypoactivation in mPFC/DLPFC is also associated with impulsivity in teenagers (Stanger et al, 2013).

The neural changes that occur during adolescence may interact synergistically with those involved in sensitization to stimulant drugs, particularly in individuals with

ADHD. For example, during adolescence, D1 receptors are overproduced in the mPFC, particularly on glutamatergic neurons that project to the nucleus accumbens (Andersen et al, 2001; Brenhouse et al, 2008; Stanis & Andersen, 2014). Altered D1 receptor activity is also associated with some of the behavioral characteristics of adolescence, such as impulsivity and novelty seeking, which are also notable in ADHD and drug users (Potvin et al, 2014; Stanis & Andersen, 2014, Tengelbeckers et al, 2015; Vonmoos et al, 2013). Moreover, as reviewed above, D1 receptors in the nucleus accumbens are critical for stimulant sensitization (De Vries et al, 1998; Henry & White, 1991; Pierce et al, 1996; Steketee & Kalivas, 2011).

Monoamine transporters are also differentially expressed throughout development. In the frontal cortex, serotonin transporter (SERT) levels increase steadily from early adolescence through old age (Moll et al, 2000). NET density in frontal cortex decreases from early to late adolescence, but thereafter remains stable throughout adulthood (Moll et al, 2000). In contrast, DAT density increases in the striatum from early adolescence until peaking in late adolescence. Thereafter, striatal DAT density declines throughout adulthood (Moll et al, 2000). As reviewed above, DAT is a critical mediator of sensitization to stimulants in reward-associated regions (Martin et al, 2011). DAT is also elevated in individuals with ADHD and those abusing cocaine (Fusar-Poli et al, 2012; Hesse et al, 2009; Mash et al, 2002; Moll et al, 2000). Chronic exposure to methylphenidate as a treatment for ADHD during the teenage years, when D1 receptor and DAT levels are at their highest, may therefore result in greater vulnerability to sensitization and later cocaine abuse risk, compared to juveniles or adults. It is worth

noting that clinically relevant doses of methylphenidate do not induce locomotor sensitization (Kuczenksi & Segal, 2002). However, this does not translate to a lack of neurochemical effects or consequences for cocaine abuse risk. Rather, the neurochemical adaptations associated with adolescent methylphenidate treatment closely resemble those associated with stimulant sensitization. For example, chronic methylphenidate exposure increases dendritic spine density in the nucleus accumbens, particularly on D1-receptor expressing medium spiny neurons, and also may increase DAT levels in striatum or PFC (Fusar-Poli et al, 2012; Kim et al, 2009; Somkuwar et al, 2013b).

Despite these observations, there are currently no well-controlled studies on methylphenidate initiation after age 13 in individuals with ADHD. Evaluation of long-term effects of adolescent methylphenidate on SUD risk in human populations has been complicated by difficulty in controlling certain variables across patients, such as the presence of conduct disorder, the precise age at which treatment was initiated, and the prescription of mixed medications, which may convey varying degrees of risk (Steinhausen & Bisgaard, 2014). Fortunately, preclinical animal models using carefully controlled experimental conditions can help address these clinically relevant questions in ADHD.

The Spontaneously Hypertensive Rat Model of ADHD

Preclinical models can begin to address important gaps in the clinical literature regarding the long-term consequences of adolescent ADHD medication for SUD and particularly cocaine abuse risk. However, studies in outbred animals have revealed

somewhat conflicting findings. For example, methylphenidate exposure during the rat juvenile period had no effect on cocaine conditioned place preference, but increased cocaine intake during self-administration in adulthood (Crawford et al, 2011), in contrast to observations in the clinical literature. Conversely, exposure to methylphenidate during the rat adolescent period reduced cocaine conditioned place preference in adulthood (Adriani et al, 2006; Andersen et al, 2001; Carlezon et al, 2003), and also decreased high-dose cocaine self-administration (1 mg/kg/infusion, Thanos et al, 2007), but increased low-dose cocaine self-administration (0.075 mg/kg/infusion, Brandon et al, 2001). These apparently conflicting findings emphasize the need to study cocaine abuse risk in an appropriate animal model, which exhibits a behavioral and neurobiological profile that mirrors observations in individuals with ADHD. Outbred animals, in contrast, are presumably more representative of the general population.

The Spontaneously Hypertensive Rat (SHR) is the most well-validated and frequently studied animal model of ADHD (Kantak et al, 2008; Russell et al, 2005; Sagvolden et al, 2005). SHR exhibit core symptoms of ADHD, including hyperactivity, impulsivity, and inattention (Adriani et al, 2003; Bayless et al, 2015; Hill et al, 2012; Sagvolden, 2000), inhibitory control and working memory deficits (Bayless et al, 2015; Kantak et al, 2008), as well as poor behavioral flexibility (Harvey et al, 2013), and increased novelty seeking (dela Peña et al, 2015). Cognitive dysfunction in SHR is most evident under difficult task conditions, such as when a delay is imposed between a correct response and delivery of reward, consistent with observations in humans with ADHD (Aase & Sagvolden, 2006; Harvey et al, 2013; Martinussen et al, 2005).

Moreover, these deficits are unrelated to hypertension, as SHR show ADHD-like symptoms even at pre-hypertensive ages (Russell et al, 2005; Watanabe et al, 1997), and anti-hypertensive treatment does not improve cognitive functioning in the SHR (Wyss et al, 2003). The Wistar-Kyoto rat (WKY), which is the progenitor strain of the SHR, does not exhibit hyperactivity, impulsivity, or hypertension, and is frequently used as an inbred control strain (Russell et al, 2005), while the Wistar rat (WIS), a common ancestor to both SHR and WKY, is used as an outbred control strain (dela Peña et al, 2012). Concurrent evaluation of these three strains is necessary to control for the genetic homogeneity of the SHR (using the WKY), while still representing the genetic heterogeneity of the general population (using the WIS).

Importantly, clinically relevant doses of ADHD medications alleviate ADHD-related symptoms in SHR. For example, in adolescent SHR, methylphenidate treatment improved the speed to learn visual cue discrimination and initial set formation, and reduced perseverative and regressive errors in attentional and strategy set shifting tasks, which are analogous to the Wisconsin Card Sorting Task in humans (Cao et al, 2012; Floresco et al, 2008, Harvey et al, 2011; 2013). The predictive validity of the SHR likely relates to the genetic and neurochemical similarities that SHR share with ADHD patients. For example, both individuals with ADHD and the SHR exhibit altered frontostriatal connectivity (Cubillo et al, 2012; Rubia, 2011; Warton et al, 2009), and elevated glutamate and dopamine release in PFC and striatum (Courvoisie et al, 2004; MacMaster et al, 2003; Miller et al, 2014). Moreover, as reviewed above, ADHD is associated with specific polymorphisms in the DAT-1 gene, (Faraone et al, 2005; Fusar-Poli et al, 2012),

and variation in the DAT-1 gene has also been observed in the SHR, which is 94% homologous to the human DAT-1 gene (Mill et al, 2005). SHR also exhibit increased binding and expression of DAT in the striatum and substantia nigra/VTA (Roessner et al, 2010; Watanabe et al, 1997), consistent with observations in human patients (Faraone et al, 2005; Fusar-Poli et al, 2012).

In addition to exhibiting cognitive and neurochemical deficits associated with ADHD, the SHR is also a compelling model for comorbid ADHD and cocaine abuse. In cocaine self-administration studies, SHR acquire lever pressing for cocaine faster, earn more cocaine infusions, are more sensitive to the reinforcing value of cocaine, and work harder to earn cocaine reinforcement than both WKY and WIS control strains (Harvey et al, 2011; Jordan et al, 2014; Somkuwar et al, 2013a). These findings indicate that the SHR may also be useful in evaluating the long-term consequences of adolescent ADHD medication on cocaine abuse risk in adulthood. Consistent with clinical studies (Mannuzza et al, 2008), adolescent treatment with a clinically relevant dose of oral methylphenidate further enhanced the speed to acquire cocaine self-administration, and further increased the efficacy and motivating influence of cocaine reinforcement in SHR, but did not increase self-administration behaviors in WKY or WIS control strains (Baskin et al, 2015; Harvey et al, 2011; Jordan et al, 2014). The same adolescent methylphenidate treatment regimen was found to increase DAT function in the mPFC of adult SHR, relative to untreated SHR and methylphenidate-treated WKY and WIS (Somkuwar et al, 2013b). Although cortical DAT function in ADHD has not been reported, these findings in the SHR model are consistent with observations in humans, as individuals with ADHD

who are treated with stimulant medications exhibit increased DAT levels compared to medication-naïve patients (Fusar-Poli et al, 2012). An increase in DAT function in mPFC may lead to faster clearance of dopamine and lower basal dopaminergic tone in mesocortical neurons (Zahniser & Sorkin, 2004). When cocaine is self-administered under these conditions, post-synaptic responses to phasically released dopamine would therefore be increased (Grace, 2001), leading to a greater reinforcing effect of cocaine in adult SHR that were treated with methylphenidate during adolescence. Importantly, these observations were not necessarily a result of discontinuing methylphenidate in adulthood. Adult SHR continued to show elevated motivation to self-administer cocaine when methylphenidate treatment was continued, as long as methylphenidate was administered after cocaine self-administration sessions (Baskin et al, 2015). However, when methylphenidate treatment was administered 1 hour prior to cocaine self-administration sessions, SHR did not show increases in cocaine intake compared to vehicle-treated SHR (Baskin et al, 2015). Methylphenidate pretreatment may block cocaine's ability to bind to DAT, by competing at the same binding site (Berglund et al, 2013; Volkow et al, 1995). Therefore, administration of methylphenidate prior to cocaine self-administration sessions may mask elevated cocaine abuse risk in adult SHR (Baskin et al, 2015; Schenk & Izenwasser, 2002). These observations suggest that if an individual with ADHD does not properly adhere to their methylphenidate treatment regimen, they may remain at increased risk of cocaine abuse, if cocaine is sampled (Baskin et al, 2015).

Taken together, findings in SHR support human studies suggesting that initiation of methylphenidate treatment in adolescence increases cocaine abuse risk in adulthood

(Dalsgaard et al, 2014; Lambert et al, 1998; Manuzza et al, 2008; Somkuwar et al, 2013b; Steinhausen & Bisgaard, 2014). The effects of adolescent methylphenidate treatment may be due to sensitization-related neurochemical changes induced by methylphenidate that persist even after treatment is discontinued. It is therefore critical to identify alternative treatments for newly diagnosed teenagers with ADHD, who may be at increased risk for substance abuse later in life if treated with methylphenidate.

CHAPTER TWO.

Aim 1: Treatment with a Non-Stimulant ADHD Medication During Adolescence and Cocaine Abuse Risk and Cocaine Cue Reactivity in Adulthood

Introduction

One potential alternative to methylphenidate treatment for adolescents with ADHD is the non-stimulant drug, atomoxetine (Strattera®). Unlike methylphenidate, atomoxetine has a very low affinity for DAT (Heal et al, 2008). Atomoxetine acts by selectively inhibiting reuptake at NET, resulting in increased extracellular concentrations of norepinephrine (Byamster et al, 2002). In addition, because dopamine uptake in the PFC is primarily by NET (Morón et al, 2002), atomoxetine increases extracellular concentrations of both norepinephrine and dopamine in this region (Bymaster et al, 2002). However, because atomoxetine does not increase dopamine transmission in the

striatum, it has low abuse potential, unlike methylphenidate (Wilens, 2006). Atomoxetine has moderately comparable efficacy to methylphenidate in relieving ADHD symptoms (Kratochvil et al, 2002), but may be more efficacious in certain subpopulations of ADHD, such as those with comorbid anxiety, in whom methylphenidate may worsen certain symptoms (Adler et al, 2009; Geller et al, 2007; Goez et al, 2007). Regionally, atomoxetine increases activation in the right IFG of healthy volunteers (Chamberlain et al, 2009), and normalizes DLPFC hypoactivation in boys with ADHD (Cubillo et al, 2014). However, atomoxetine may not be as efficacious as methylphenidate in mediating VLPFC function or in improving performance on discrimination tasks (Cubillo et al, 2014; Smith et al, 2013). Similar to methylphenidate, increased stimulation at dopamine D1 and alpha2A noradrenergic receptors is thought to underlie atomoxetine's cognitive-enhancing effects (Arnsten & Pliszla, 2011). It is worth noting here that guanfacine (Intuiv®) is another non-stimulant medication for ADHD, which acts as an agonist at alpha2a noradrenergic receptors (Nakagawa et al, 1982; Wilens, 2006). However, guanfacine is more often prescribed as an adjunct therapy to stimulant medications, and has limited efficacy in adolescents with ADHD (Sibley et al, 2011). Atomoxetine is therefore the focus of Aim 1.

In adolescent SHR, a pharmacologically relevant dose of atomoxetine improves behavioral flexibility during the set shift phase of the strategy set shifting task (Harvey et al, 2013). Behavioral flexibility during the set shift phase is a process that is dependent on mPFC function (Floresco et al, 2008). Atomoxetine also progressively and dose-dependently reduces hyperactivity in young adult SHR over the course of 21 days of

treatment (Moon et al, 2014). Unlike methylphenidate, adolescent treatment with atomoxetine does not alter DAT function in mPFC in adulthood, but rather decreases DAT function and cell surface distribution in the OFC of adult SHR (Somkuwar et al, 2013a). Thus, atomoxetine-induced improvement in set shifting, a mPFC-mediated process, cannot be explained by a change in DAT function in SHR. A decrease in NET function in mPFC of SHR could explain such an improvement, as previous research in outbred rats has shown improvement in set shifting performance after activation of alpha1 noradrenergic receptors in mPFC (Lapiz & Morilak, 2006) and impairment after selective noradrenergic lesions in mPFC (McGaughy et al, 2008). Interestingly, ADHD is associated with variations in the NET allele, which are related to externalizing symptoms such as impulsivity (Hohmann et al, 2015). However, additional work is needed to determine the effects of adolescent atomoxetine treatment on mPFC NET function in SHR.

The decrease in DAT function and cell surface distribution in the OFC of adult SHR may play a role in potentially protective effects of adolescent atomoxetine on cocaine abuse risk (Somkuwar et al, 2013a). Importantly, dopamine transmission in the OFC is associated with cocaine seeking (Di Pietro et al, 2008), and transient inactivation of the OFC may reduce the motivation to self-administer cocaine and alter cocaine's reinforcing value (Kantak et al, 2009). These findings suggest that decreased DAT function in OFC may lead to diminished reinforcing effects of cocaine in adult SHR that were treated with atomoxetine during adolescence. It is therefore possible that adolescent atomoxetine treatment may not increase cocaine abuse risk in adulthood, in contrast to

previous observations with methylphenidate. In adult outbred rats, acute atomoxetine administration reduced cocaine seeking (Economidou et al, 2011; Janak et al, 2012). However, the effects of chronic adolescent atomoxetine treatment on cocaine abuse risk in an animal model of ADHD have not yet been examined. Experiments 1 through 3 address this clinically important question by evaluating the speed of acquisition of cocaine self-administration, and the efficacy and motivating influence of cocaine reinforcement, in adult rats following discontinuation of adolescent atomoxetine treatment. Importantly, treatment cessation is common in young adults with ADHD (McCarthy et al, 2009; Zetterqvist et al, 2012).

In addition to cocaine abuse risk resulting directly from the reinforcing value of cocaine, the environmental cues associated with cocaine use play a major role in the development of compulsive drug seeking and relapse behaviors. Although it is unknown whether ADHD influences reactivity to drug-related cues, cigarette smokers with ADHD report more frequent encounters with smoking-associated cues and perceive a stronger relationship between cues and smoking motivation than smokers without ADHD (Mitchell et al, 2013). These findings suggest that drug-related cues may be more salient to individuals with ADHD. In cocaine-dependent subjects, videos involving the preparation or consumption of cocaine elicit greater activation in regions also known to be dysfunctional in ADHD, including the OFC and DLPFC, in comparison to healthy controls or videos involving the preparation or consumption of food (Wilcox et al, 2011). Similarly, in non-human primates, a visual stimulus associated with the reinforcing value of cocaine was found to increase activation in ventral OFC and VLPFC compared to a

control stimulus, and this activation was positively correlated with increasing preference for the cocaine-paired stimulus over the control stimulus (Nelissen et al, 2012). Because individuals with ADHD exhibit elevated OFC activation during reward-based tasks (von Rhein et al, 2015), exposure to cocaine and cocaine-related cues may exacerbate OFC dysfunction. This may lead to elevated cue reactivity and an increased risk of relapse in individuals with ADHD, given that cortical activation to cocaine cues is associated with treatment dropout and higher relapse rates in cocaine dependent patients (Kosten et al, 2005).

The environmental cues associated with cocaine use are also linked to changes in dopamine-mediated transmission in cortical sites involved in ADHD. For example, systemic administration of a dopamine D1 receptor antagonist not only attenuated cocaine seeking behavior, but also reversed cocaine cue-induced increases in Fos expression in the basolateral amygdala and mPFC (Ciccocioppo et al, 2001), and infusion of D1 antagonists directly into the OFC reduced cocaine intake and cocaine seeking (Di Pietro et al, 2008). Notably, as reviewed above, DAT function in the mPFC and OFC is affected by adolescent ADHD medications (Somkuwar et al, 2013a, b). Previous work examining cue reactivity following adolescent methylphenidate treatment showed that prior methylphenidate exposure increased cocaine intake in adult SHR, and neither promoted nor protected against increased cocaine cue reactivity or relapse behaviors (Jordan et al, 2014). However, in outbred rats, acute atomoxetine infusions reduced cue-induced cocaine seeking, both when cocaine was and was not available for self-administration (Economidou et al, 2011; Janak et al, 2012). These findings suggest that

NET, the primary target of atomoxetine, plays an important role in regulating saliency of drug-associated cues. Thus, the effects of chronic adolescent atomoxetine treatment on cocaine cue reactivity in the SHR model of ADHD must be examined. Experiment 4 addresses this clinically important question by measuring cocaine-seeking behavior under a second-order schedule of cocaine delivery and cue presentation. Cocaine seeking reflects cue reactivity (i.e., lever responding maintained by drug-paired cue presentation) and typically is measured under two experimental conditions: one condition during which cocaine is available for self-administration (maintenance testing) and another condition during which cocaine is not available for self-administration (reinstatement testing) (Kantak et al, 2002).

Experiment 1: Acquisition of Cocaine Self-Administration Following Adolescent Atomoxetine Treatment

Experiment 1 tested the hypothesis that adult SHR acquire cocaine self-administration faster, at which time more cocaine infusions would be earned and more lever responses would be made compared to adult WKY and WIS control strains. Elevation in these measures is suggestive of increased risk to rapidly develop compulsive patterns of cocaine abuse (Somkuwar et al, 2013a). Experiment 1 also tested the hypothesis that, in contrast to methylphenidate, treatment with a pharmacologically relevant dose of atomoxetine during adolescence does not further increase the speed to

acquire cocaine self-administration in adult SHR after adolescent treatment is discontinued.

Materials and Methods

Animals and Treatments. All procedures were approved by the Institutional Animal Care and Use Committee at Boston University, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Eighth Edition). Male WKY/Cr, WIS/Cr, and SHR/Cr rats (Charles River Laboratories, Wilmington, MA, Kingston, NY or Raleigh, NC) arrived on postnatal day 25 (P25). SHR served as an animal model of ADHD, and WKY and WIS as inbred and outbred comparator strains, respectively. Using data from past studies in the Kantak laboratory, power analysis (beta = 90; $p \leq 0.05$) indicated that group sizes of at least 6-7 were needed to detect statistical differences across the various measures. Final group sizes for Experiment 1 included $n = 8$ for vehicle- and atomoxetine-treated WKY as well as vehicle-treated WIS and SHR, $n = 10$ for atomoxetine-treated SHR, and $n = 11$ for atomoxetine-treated WIS. Thus, these group sizes permitted meaningful analysis and interpretation of the data.

Rats were housed individually in plastic cages in a temperature-21–23°C and light- (07:30 hours on; 19:30 hours off) controlled vivarium. Rats were not given environmental enrichment because past research has demonstrated that environmental enrichment reduces ADHD-like symptoms in the SHR strain (Pamplona et al, 2009). Rats were allowed 3 days to acclimatize to new housing conditions in the Laboratory Animal

Care Facility prior to experimental initiation (see Figure 1 for an experimental timeline). The 3-day acclimation period is standard protocol in animal studies assessing cocaine self-administration (Roberts et al, 2002), neurochemistry (Mateo et al, 2005) as well as in studies using adolescent rats (Levin et al, 2007).

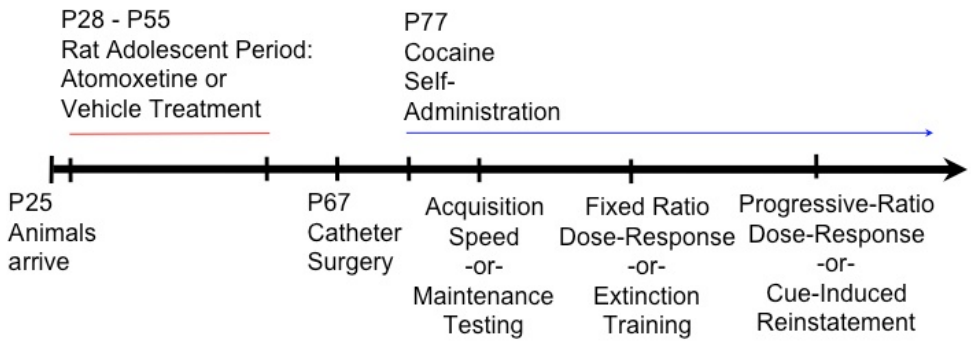

Figure 1. Timeline of experimental events in Aim 1.

From P28 through P55, constituting the rat adolescent period (Spear, 2000), animals received once daily intraperitoneal (i.p.) injections of either a pharmacologically relevant dose of atomoxetine (0.3 mg/kg) dissolved in 0.9% sterile saline, or saline alone (2 ml/kg). The double injection volume was used to enable complete dissolution of atomoxetine. Treatments were administered once daily, Monday-Friday. This regimen mimics the weekend medication "holiday" that is sometimes elected by individuals with ADHD, in part to reduce the occurrence of side effects (American Academy of Pediatrics Committee on Children With Disabilities and Committee on Drugs, 1996; Faraone et al, 2004; Martins et al, 2004). The relatively low dose of atomoxetine was chosen to increase

extracellular norepinephrine and dopamine concentrations primarily in PFC, through inhibition of NET (Bymaster et al, 2002). An i.p. route of administration was utilized due to poor bioavailability of atomoxetine in rats (Mattiuz et al, 2003). Food was restricted from P28-P55 to ~90% of a growth-adjusted free-feeding body weight to mimic conditions of past comparator studies (Harvey et al, 2011; 2013; Somkuwar et al 2013b). Animals had free access to water throughout experiments, and food was freely available after P55.

Surgery. Adolescent atomoxetine or vehicle treatment was discontinued on P56. On P67, rats were implanted with intravenous (i.v.) catheters connected to headmounts to enable cocaine self-administration. Animals were anesthetized with i.p. injections of 90 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA) and 8-10 mg/kg xylazine (Akorn, Decatur, IL). Subcutaneous (s.c.) Buprenex (0.05 mg/kg, Butler Schein, Columbus, OH) was given as a preemptive analgesic 5 minutes prior to anesthetization. Incisions were made to expose the right femoral vein and skull. A catheter constructed of silicon tubing (i.d. = 0.51, o.d. = 0.94 mm) was inserted s.c. between these incisions. The proximal end of the catheter was inserted into the right femoral vein and anchored to tissue underlying the vein using surgical silk and 0.5 mm square Teflon mesh (CR Bard Incorporated, Charlotte, NC). The distal end of the catheter was attached to a 22-gauge L-shaped pedestal mount (Plastics One, Roanoke, VA). The pedestal was secured to the skull using four stainless-steel screws and acrylic cement. The stainless steel tubing exiting the pedestal was plugged with a sealed piece of Teflon tubing and covered with a

protective plastic outer screw cap. The antibiotic Baytril (5 mg/kg, i.v.; Bayer HealthCare, Shawnee, Mission, KS) was given for up to 5 days post-surgery to reduce the risk of systemic infection, and the anti-inflammatory Meloxicam (0.3 mg/kg, s.c., Boehringer Ingelheim Vetmedica, St. Joseph, MO) for 3 days post-surgery. Buprenex (0.025 mg/kg s.c.; Bulter Schein, Columbus, OH) was given for 48 hr following surgery or as needed, and 0.9% sterile saline was administered s.c. at least once and as needed subsequently (based on body weight loss) to maintain hydration.

Catheters were maintained by once daily flushing, Monday-Friday, with 0.2 ml of 0.9% sterile saline containing heparin (30 IU /ml; Baxter Healthcare, Deerfield, IL) and Timentin (67 mg /ml; Glaxo-SmithKline, Research Triangle Park, NC). Before weekends and holidays, 0.08 ml of a locking solution consisting of glycerol (Sigma-Aldrich, St. Louis, MO) and 1000 IU/ml heparin was infused in a 3:1 ratio to fill catheter dead space and minimize the occurrence of blockages, thus prolonging the longevity of the catheter. The locking solution was removed and replaced with 0.1 ml of 0.9% sterile saline containing heparin (3 IU/ml) prior to resumption of behavioral procedures. Catheter patency was assessed weekly either by verifying the ability to withdraw blood or by observing rapid loss of muscle tone following a 0.1 ml infusion (10 mg/ml) of methohexital sodium (JHP Pharmaceuticals, Rochester, MI). In the event that patency was lost, catheters were repaired or replaced with a new catheter, implanted into the left femoral vein or the right or left jugular vein, under surgical anesthesia and post-operative care, as above. Animals were allowed to recover before resuming cocaine self-

administration experiments. As a result, no animals were excluded from statistical analyses due to loss of catheter patency.

Testing Environment. Experimental chambers were equipped with two response levers, a white stimulus light mounted above the designated active lever, and a house light on the opposite wall (model ENV-008CT, Med Associates, St Albans, VT). Chambers were also fitted with a single channel fluid swivel (Instech Solomon, Plymouth Meeting, PA) and spring leash assembly, connected to a counterbalanced arm (Med Associates). During self-administration sessions, animals were leashed within the chamber via a hexnut connection to the pedestal mount, allowing free and comfortable movement throughout the chamber. Experimental chambers were enclosed in a sound-attenuating cubicle, equipped with a fan for ventilation and an 8-ohm speaker to provide a background contextual cue consisting of white noise. The motor-driven syringe pumps (model ENV-018 M; Med Associates) used for cocaine delivery were located inside the cubicle. A PC-compatible computer programmed in Medstate Notation and connected to a Med Associates interface controlled experimental events from an adjacent room.

Cocaine Self-Administration. For intravenous self-administration, cocaine hydrochloride was dissolved in 0.9% sterile saline containing heparin (3 IU/ml). A 0.8 mg/ml solution of cocaine was infused at a rate of 1.8 ml/min. The infusion duration was adjusted for body weight (1.2 s/100 g) to attain a training dose of 0.3 mg/kg cocaine, consistent with prior studies in SHR (Harvey et al, 2011).

On P77, after 10 days of surgical recovery, animals were allowed to press the active lever (either left or right, counterbalanced across strains and treatments) for infusions of 0.3 mg/kg cocaine delivered under a fixed-ratio-1 (FR1) schedule of reinforcement. Under the FR1 schedule, each cocaine infusion required one response on the active lever and was paired with a 20-sec illumination of the stimulus light above the active lever. During this 20-sec period, the house light was extinguished and additional cocaine infusions could not be earned, though lever responses were still counted. The 20-sec timeout period also served to prevent accidental overdose. After the 20 sec elapsed, the house light was re-illuminated. Responses on the inactive lever were recorded, but had no consequences. Importantly, animals received no external inducements to respond on either lever, such that acquisition of cocaine self-administration was entirely spontaneous. Sessions (2 hr) were conducted during the light phase, once daily, Monday-Friday, at approximately the same time each day. Acquisition criterion was defined as earning ≥ 20 infusions for two consecutive sessions, and discriminating the active from inactive lever by a factor of 2 or greater (following Harvey et al, 2011). Animals were allowed a maximum of 25 sessions to spontaneously acquire cocaine self-administration, after which time lever pressing was facilitated via baiting of the active lever with a 45-mg chocolate-flavored food pellet (BioServ, Frenchtown, NJ). Baiting was necessary for one vehicle-treated WIS (32 sessions to acquire) and one atomoxetine-treated WIS (34 sessions to acquire). All other animals reached acquisition criterion in 23 sessions or less.

Data Analysis. Dependent measures in Experiment 1 included the number of sessions required to reach acquisition criterion (square root transformed prior to analysis due to non-normality), as well as cocaine infusions earned and active and inactive lever responses at acquisition criterion. Measures were analyzed by two-factor (strain X treatment) ANOVAs, followed by post-hoc Tukey tests.

Results

Sessions to reach the acquisition criterion for the 0.3 mg/kg training dose of cocaine are shown in Figure 2. Strains differed significantly ($F(2, 47) = 7.0, p \leq 0.002$), and there was a trend for a strain X treatment interaction ($F(2, 47) = 2.7, p \leq 0.07$). Overall, SHR acquired cocaine self-administration faster than WKY and WIS ($p \leq 0.04$ and 0.002, respectively). Strain comparisons within each treatment revealed that in vehicle-treated rats, SHR acquired cocaine self-administration faster than WKY ($p \leq 0.02$). In atomoxetine-treated rats, both SHR and WKY acquired cocaine self-administration faster than WIS ($p \leq 0.01$ and 0.05, respectively). Treatment comparisons within each strain revealed that in WKY, acquisition of cocaine self-administration was faster after atomoxetine than vehicle ($p \leq 0.03$). Notably, atomoxetine did not alter acquisition speed in SHR or in the WIS control.

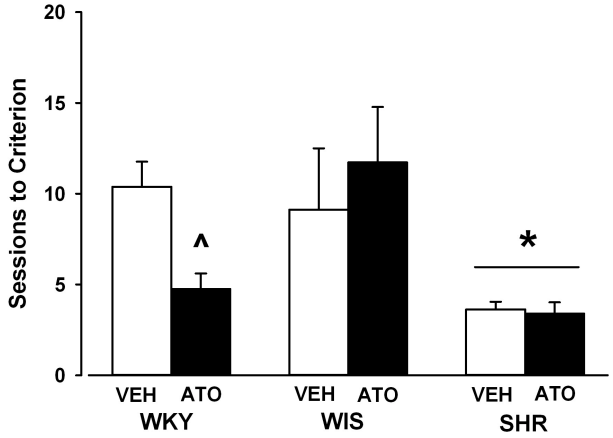

Figure 2. Number of sessions required to reach acquisition criterion in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

The number of cocaine infusions earned at the acquisition criterion for the 0.3 mg/kg training dose of cocaine is shown in Figure 3. Strains differed significantly ($F(2, 47) = 6.2$, $p \leq 0.004$), but the treatment factor and its interaction with strain were not significant. Post-hoc comparisons showed that, overall, SHR earned more cocaine infusions than WKY ($p \leq 0.003$), and there was a trend towards more infusions compared to WIS ($p \leq 0.09$).

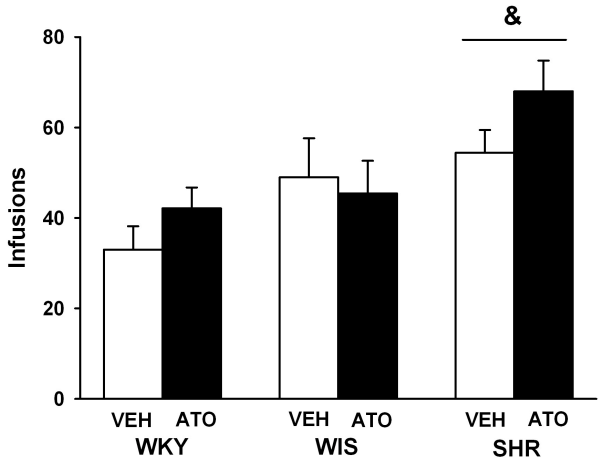

Figure 3. Number of cocaine infusions earned at acquisition criterion in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). & $p \leq 0.05$ compared to WKY overall.

Active lever responses at criterion are shown in Figure 4. Strains again differed significantly ($F(2, 47) = 4.5$, $p \leq 0.02$), and there was a trend towards a strain X treatment interaction ($F(2, 47) = 2.7$, $p \leq 0.08$). Post-hoc comparisons showed that, overall, SHR made more active lever responses than WKY ($p \leq 0.02$), and there was a trend towards more active lever responses than WIS ($p \leq 0.07$). Treatment comparisons within each strain revealed that in adult SHR, active lever responses were greater after adolescent atomoxetine than vehicle ($p \leq 0.006$). Strain comparisons within each treatment condition revealed that in atomoxetine-treated rats, SHR made more active lever responses than WKY and WIS ($p \leq 0.003$ and 0.006, respectively). There were no strain differences in active lever responses among vehicle-treated rats at criterion. With respect to inactive lever responses, there were no strain or treatment differences at

criterion, and each strain discriminated the active from inactive lever by a factor of 2 or greater (9:1 for WKY, 20:1 for WIS, and 4:1 for SHR, on average).

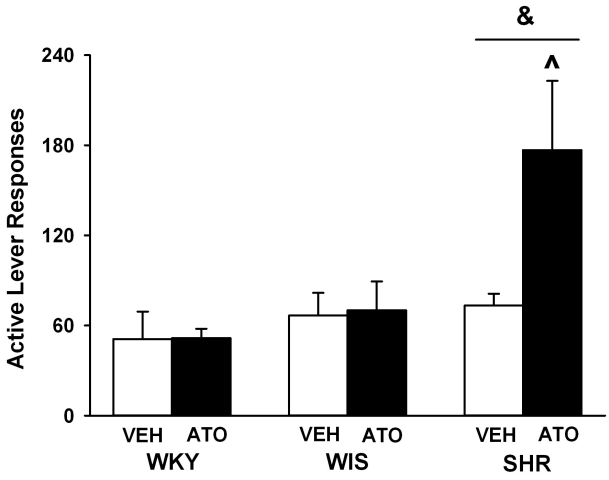

Figure 4. Number of active lever responses made at acquisition criterion in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). & $p \leq 0.05$ compared to WKY overall. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Experiment 2: Fixed-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent Atomoxetine Treatment

Experiment 2 tested the hypothesis that the efficacy of cocaine reinforcement is elevated in adult SHR compared to WKY or WIS, such that SHR earn more cocaine infusions and make more responses on the active lever across a range of doses under a FR1 schedule of reinforcement. Experiment 2 also tested the hypothesis that, in contrast

to the effects of methylphenidate, treatment with a pharmacologically relevant dose of atomoxetine during adolescence does not increase the efficacy of cocaine reinforcement in adult SHR after adolescent treatment is discontinued.

Materials and Methods

Animals and Treatments. Following achievement of the acquisition criterion in Experiment 1, the same groups of animals were allowed to continue responding under an FR1 schedule for the 0.3 mg/kg training dose, until active lever responses and the number of cocaine infusions varied ≤ 15% for 5 consecutive sessions. A range of cocaine unit doses (0.003, 0.01, 0.03, 0.1, and 1.0 mg/kg/infusion) was then substituted in place of the training dose in a pseudo-random order, every Tuesday and Friday. The 0.3 mg/kg training dose was available on the intervening days (Monday, Wednesday, and Thursday). As above, sessions were 2 hours in length and were conducted during the light phase once daily, Monday-Friday, at approximately the same time each day. After the full FR cocaine dose-response functions were completed, rats were allowed to return to baseline responding for the 0.3 mg/kg training dose. Two atomoxetine-treated WIS and two atomoxetine-treated SHR expired prior to completion of Experiment 2, and were thus excluded from Experiment 2 data analyses.

Data Analysis. Dependent measures in Experiment 2 included the number of cocaine infusions earned, and active and inactive lever responses for each cocaine dose.

Measures were analyzed by three-factor (strain X treatment X dose) ANOVAs, with repeated measures for dose, followed by post-hoc Tukey tests.

Results

Cocaine dose-response functions based on the number of infusions earned under the FR1 schedule of reinforcement are shown in Figure 5. For the three-way ANOVA, strain ($F(2, 43) = 18.4$, $p \leq 0.001$) and dose ($F(5, 215) = 185.5$, $p \leq 0.001$) differed, and there was also a strain X dose interaction ($F(10, 215) = 9.3$, $p \leq 0.001$). The treatment factor and its interactions with strain and/or dose were not significant, indicating that adolescent atomoxetine treatment did not further increase cocaine intake in SHR or in control strains. Overall, SHR earned more cocaine infusions than WKY and WIS ($p \leq 0.001$). Further testing of the strain X dose interaction indicated that SHR earned more infusions than WKY for cocaine doses ranging from 0.003 to 0.3 mg/kg (ps ≤ 0.04), and more infusions than WIS for cocaine doses ranging from 0.003 to 0.1 mg/kg (ps ≤ 0.01, except at 0.01mg/kg, where $p \leq 0.08$). In addition, WIS earned more infusions than WKY at 0.1 mg/kg ($p \leq 0.001$). No strain differences were observed at 1.0 mg/kg.

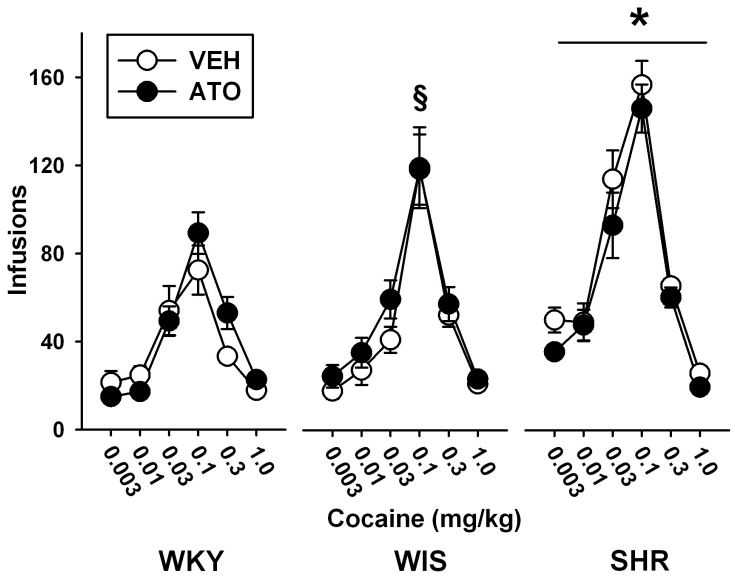

Figure 5. Cocaine dose-response functions based on infusions earned under a FR1 schedule of reinforcement by adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. § $p \leq 0.05$ compared, overall, to the same dose in WKY.

Analyses of the cocaine dose–response functions based on the number of active lever responses (Figure 6) were similar to the number of infusions earned. For the three-way ANOVA, strain ($F(2, 43) = 4.9$, $p \leq 0.01$) and dose ($F(5, 215) = 36.9$, $p \leq 0.001$) differed significantly, and there was a strain X dose interaction ($F(10, 215) = 3.2$, $p \leq 0.001$). The treatment factor and its interactions with strain and/or dose were not significant, indicating that adolescent atomoxetine treatment did not further increase cocaine-maintained responding in SHR or in control strains. Post-hoc comparisons showed that SHR overall made more active lever responses than WKY ($p \leq 0.01$), but SHR were not different from WIS. Further testing of the strain X dose interaction revealed that SHR made more active lever responses at the 0.03 and 0.1 mg/kg doses than

WKY and WIS (*p*s ≤ 0.05). Furthermore, WIS made more active lever responses at 0.1 mg/kg than WKY ($p \leq 0.003$). No strain differences at the other doses were found. There were no strain or treatment differences in inactive lever responding.

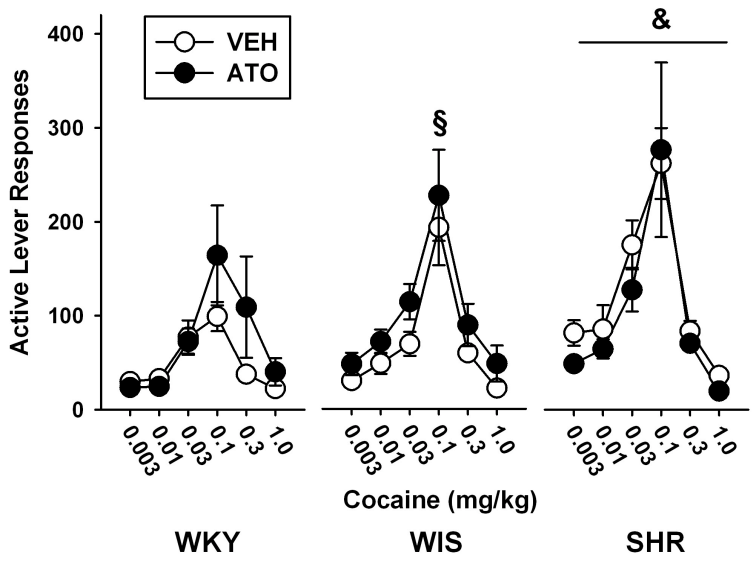

Figure 6. Cocaine dose-response functions based on active lever responses made under a FR1 schedule of reinforcement by adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). & $p \leq 0.05$ compared to WKY overall. § $p \leq 0.05$ compared, overall, to the same dose in WKY.

Experiment 3: Progressive-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent Atomoxetine Treatment

Experiment 3 tested the hypothesis that the motivating influence of cocaine reinforcement is elevated in adult SHR compared to WKY or WIS, such that SHR work

harder to earn cocaine reinforcement, earn more cocaine infusions and make more active lever responses across a range of doses under a progressive ratio (PR) schedule. Experiment 3 also tested the hypothesis that, in contrast to methylphenidate, treatment with a pharmacologically relevant dose of atomoxetine during adolescence does not increase the motivating influence of cocaine reinforcement in adult SHR after adolescent treatment is discontinued.

Materials and Methods

Animals and Treatments. Once rats returned to baseline levels of responding for 0.3 mg/kg cocaine under the FR1 schedule in Experiment 2, a PR schedule of reinforcement was instituted in the same groups of animals. The PR schedule involved a geometric increment in the number of responses required to earn each cocaine infusion (e.g., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc., following Loh & Roberts, 1990). Sessions were terminated when a rat failed to reach the next response requirement within 1 hour of the previous infusion. The PR breakpoint was defined as the last FR response requirement completed to earn a cocaine infusion. Animals were allowed to respond for 0.3 mg/kg cocaine under the PR schedule until breakpoints, cocaine infusions, and active lever responses varied ≤ 15% for 5 consecutive sessions. Cocaine test doses were then substituted (0.01, 0.1, 0.3, and 1.0 mg/kg) in descending order (following Harvey et al, 2011), with 2-3 consecutive sessions allocated to each test dose. Baseline responding was reestablished for the 0.3 mg/kg dose after the full PR dose-response function was completed to ensure that PR baseline

performance was stable after dose-substitution testing. As above, self-administration sessions were conducted during the light phase once daily, Monday-Friday, at approximately the same time each day. One atomoxetine-treated WIS expired prior to completion of Experiment 3, and was thus excluded from Experiment 3 data analyses. At the end of Experiment 3, all animals were humanely euthanized with an overdose of sodium pentobarbital (Euthasol).

Data Analysis. Dependent measures in Experiment 3 included PR breakpoints, the number of cocaine infusions earned, and active and inactive lever responses for each cocaine dose. Measures were analyzed by three-factor (strain X treatment X dose) ANOVAs, with repeated measures for dose, followed by post-hoc Tukey tests.

Results

Cocaine dose-response functions based on the PR breakpoint are shown in Figure 7. For the three-way ANOVA, strain ($F(2, 42) = 10.1$, $p \leq 0.001$) and dose ($F(3, 126) = 53.3$, $p \leq 0.001$) differed. The treatment factor and its interactions with strain and/or dose were not significant, indicating that adolescent atomoxetine treatment did not further increase the motivating influence of cocaine in SHR or in control strains. Overall, SHR reached higher breakpoints than WKY and WIS ($p \leq 0.001$ and 0.01, respectively). Further analysis of the dose factor revealed that animals maintained the highest breakpoints at 1.0 mg/kg, which differed from all other doses, and maintained the lowest

breakpoints at 0.01 mg/kg, which also differed from all other doses (*ps* ≤ 0.001 - 0.03). Breakpoints maintained by 0.3 and 0.1mg/kg did not differ from each other.

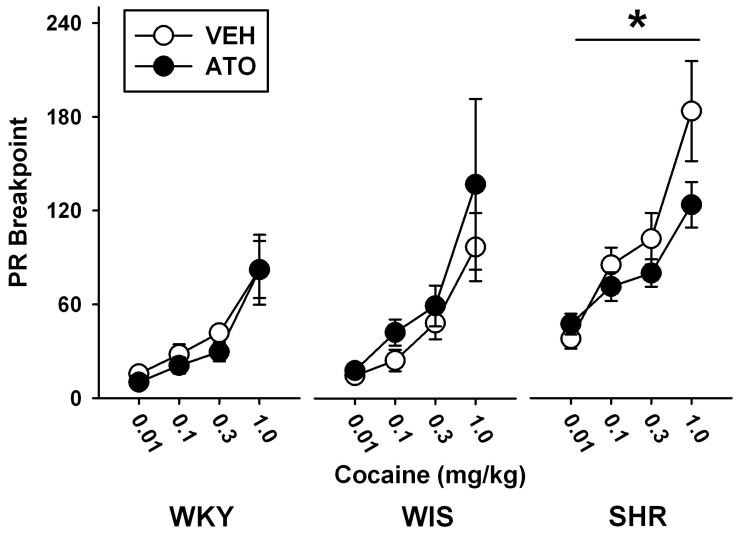

Figure 7. Cocaine dose-response functions, based on breakpoints reached under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

The PR cocaine dose-response functions based on the number of infusions earned are shown in Figure 8. For the three-way ANOVA, strain ($F(2, 42) = 17.1$, $p \leq 0.001$) and dose differed significantly ($F(3, 126) = 158.4$, $p \leq 0.001$). The treatment factor and its interactions with strain and/or dose were not significant, indicating that adolescent atomoxetine treatment did not further increase cocaine intake in SHR or in control strains. Post-hoc tests showed that, overall, SHR earned more cocaine infusions than WKY and WIS ($p \leq 0.001$). Further analysis of the dose factor showed that animals earned the greatest number of cocaine infusions at the 1.0 mg/kg dose compared to the

three other doses (*p*s ≤ 0.001), and earned the fewest cocaine infusions at the 0.01 mg/kg dose compared to the three other doses (*p*s ≤ 0.001). In addition, more cocaine infusions were earned at the 0.3 mg/kg dose than at the 0.1 mg/kg dose ($p \leq 0.001$).

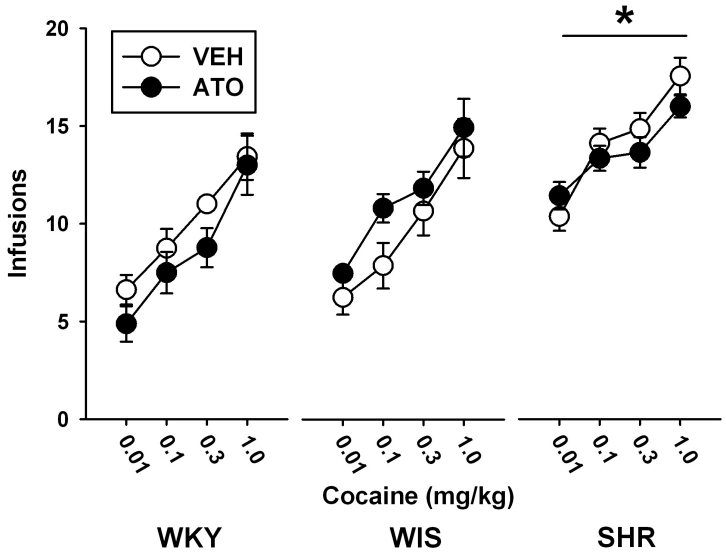

Figure 8. Cocaine dose-response functions, based on infusions earned under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

The PR cocaine dose-response functions based on active lever responses are shown in Figure 9. For the three-way ANOVA, strain ($F(2, 42) = 10.5$, $p \leq 0.001$) and dose ($F(3, 126) = 51.5$, $p \leq 0.001$) differed significantly. The treatment factor and its interactions with strain and/or dose were not significant, indicating that adolescent atomoxetine treatment did not further increase cocaine-maintained responding in SHR or

in control strains. Post-hoc testing showed that, overall, SHR made more active lever responses than WKY and WIS ($p \leq 0.001$ and $p \leq 0.008$, respectively). Further analysis of the dose factor revealed that animals made the most active lever responses for the 1.0 mg/kg cocaine dose, compared to the three other doses (ps ≤ 0.001), and the fewest active lever responses for 0.01 mg/kg cocaine dose, compared to the three other doses (ps $\leq$ 0.001, except at the 0.1 mg/kg dose where $p \leq 0.05$). Active lever responding for the two intermediate cocaine doses, 0.3 mg/kg and 0.1 mg/kg, did not differ from each other. There were no strain or treatment differences in inactive lever responding.

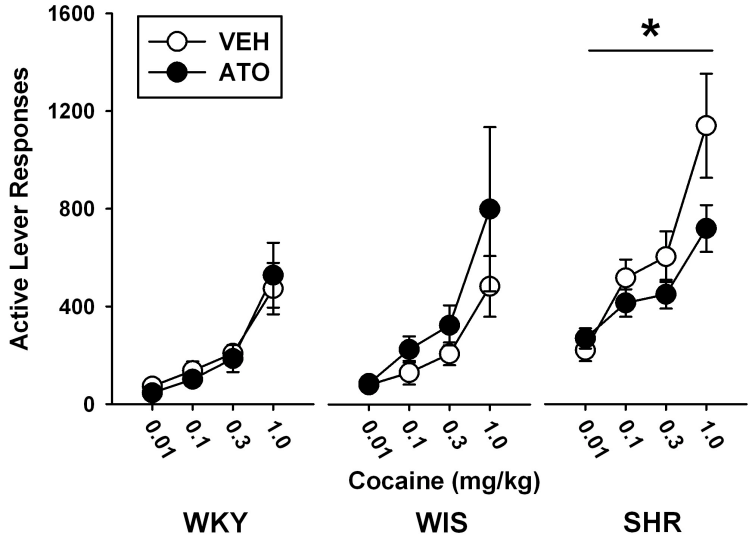

Figure 9. Cocaine dose-response functions, based on active lever responding under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Experiment 4: Cocaine Cue Reactivity and Relapse Behavior in Adulthood Following Adolescent Atomoxetine Treatment

Experiment 4 tested the hypothesis that adult SHR are most reactive to cocaine-related cues, such that SHR would emit more cocaine-seeking responses than WKY and WIS under a second-order schedule. Experiment 4 also tested the hypothesis that treatment with a pharmacologically relevant dose of atomoxetine during adolescence does not increase reactivity to cocaine cues in adult SHR after adolescent treatment is discontinued.

Materials and Methods

New groups of SHR, WKY and WIS were used in Experiment 4. The housing, treatments, surgical procedures, and testing environments were identical to those detailed in Experiment 1.

Experiment 4a: Maintenance Testing under a Second-Order Schedule. On post-natal day 76, following recovery from surgical implantation of the i.v. catheter, rats were allowed to lever press for 45-mg chocolate-flavored food pellets (BioServ, Frenchtown, NJ) under a FR1 schedule to facilitate acquisition of lever responding. The next day, rats began self-administration training for 0.3 mg/kg cocaine under an FR1 schedule. This dose was selected because it produces the highest rate of responding under a second-

order schedule of cocaine delivery in rats (Kantak et al, 2009). This dose also was used previously to examine the effects of adolescent methylphenidate treatment on cocaine self-administration behavior studied under a second-order schedule (Jordan et al, 2014). Sessions were initiated with illumination of the house light and onset of white noise. Cocaine infusions under the FR1 schedule coincided with 20-sec presentation of the stimulus light, while the house light was extinguished. During this period, additional cocaine infusions could not be earned, to reduce risk of accidental overdose. The house light was re-illuminated and the stimulus light extinguished after the 20-sec timeout period. Rats were trained incrementally to a terminal fixed-interval (FI) based second-order schedule, designated FI 5-min [FR5 : S]. Under this schedule, every fifth lever press (FR5) produced a 2-sec cue light, and the first FR5 response unit completed after the 5-min FI elapsed resulted in a cocaine infusion coinciding with 20-sec presentation of the stimulus light, after which the FI 5-min component was again in effect. Responses on the inactive lever were also recorded throughout the session, but had no consequences. Self-administration sessions were 2 hours in length and were conducted during the light phase once daily, Monday-Friday, at approximately the same time each day. Training under the second-order schedule continued until rats reached stable levels of responding (≤ 15% variation in active lever responding and ≤ 33% of total responses on the inactive lever) for a minimum of 5 sessions. This stable baseline period served as the maintenance testing phase. Final group sizes for all phases of Experiment 2 included $n = 8$ for vehicle- and atomoxetine-treated WKY and SHR as well as atomoxetine-treated WIS, and $n = 9$ for vehicle-treated SHR.

Experiment 4b: Extinction Training. Following completion of the maintenance testing phase, rats underwent response extinction training, to reduce the learned association between lever responding and cocaine reinforcement, while leaving the association between cocaine-related cues and cocaine reinforcement intact. During this phase, the house light was illuminated throughout the session, but the cue light and white noise were not presented, and cocaine infusions could not be earned. Active and inactive lever responses therefore had no consequences. Extinction training continued for a minimum of 10 sessions or until criterion was reached, defined as active lever responding that was ≤ 10% of the maintenance baseline for 3 consecutive sessions (constituting the extinction baseline). If rats did not reach criterion, a maximum of 21 extinction sessions was imposed. As above, sessions were 2 hours in length and were conducted during the light phase once daily, Monday-Friday.

Experiment 4c: Cue-Induced Reinstatement Testing. Following achievement of extinction criterion, rats underwent cue-induced reinstatement testing to assess the ability of cocaine-paired cues to induce relapse to previously extinguished cocaine-seeking behavior. Relapse to cocaine seeking is shown by a significantly greater number of active lever responses during the reinstatement test relative to the extinction baseline. During this phase, the discrete stimulus light and contextual white noise cues were presented under contingencies identical to self-administration training under the second-order schedule, but cocaine infusions could not be earned. Reinstatement testing sessions were 1 hour in duration and were conducted during the light phase once daily, Monday-Friday.

All rats underwent 7 reinstatement sessions. At the end of Experiment 4c, all animals were humanely euthanized with an overdose of sodium pentobarbital (Euthasol).

Data Analysis. Dependent measures in Experiment 4 included the number of cocaine infusions earned, active and inactive lever responses, and the number of sessions required to reach extinction criterion. Measures were analyzed by three-factor (strain X treatment X reinstatement session, or strain X treatment X phase) ANOVAs, with repeated measures for session or phase (extinction vs. reinstatement), followed by post-hoc Tukey tests.

Results

Experiment 4a: Maintenance Testing under a Second-Order Schedule. Cocaine intake during maintenance testing under the second-order schedule is shown in Figure 10. Strains differed in number of cocaine infusions ($F(2, 42) = 16$, $p \leq 0.001$), with adult SHR earning more infusions than WKY and WIS ($p \leq 0.001$). Main and interaction effects of treatment were not significant, and further analysis confirmed that adolescent atomoxetine did not significantly alter cocaine intake compared to vehicle treatment in any strain during maintenance testing.

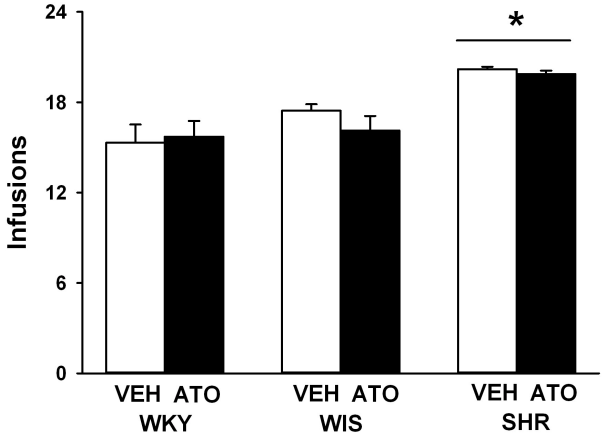

Figure 10. Cocaine intake during maintenance testing under a second-order schedule of reinforcement in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Active lever responses during maintenance testing and during the first drug-free interval of the final maintenance testing session (i.e., the first 5-min FI of the session occurring prior to delivery of the initial cocaine infusion) are shown in Figures 11 and 12, respectively. During maintenance testing, strains differed ($F(2, 42) = 29.6$, $p \leq 0.001$), with adult SHR making more active lever responses than WKY and WIS ($p \leq 0.001$). During the first drug-free interval, there was a main effect of strain ($F(2, 42) = 31.4$, $p \leq 0.001$) and a strain X treatment interaction ($F(2, 42) = 4.1$, $p \leq 0.02$). Overall, adult SHR made more active lever responses than WKY and WIS during the first drug-free interval ($p \leq 0.001$). Post-hoc testing of the interaction revealed that adolescent atomoxetine reduced active lever responses compared to vehicle treatment in adult SHR during the first drug-free interval ($p \leq 0.005$). In contrast, adolescent atomoxetine did not

significantly alter active lever responses compared to vehicle treatment during the first drug-free interval in WKY or WIS. There were no significant strain or treatment differences in inactive lever responding during maintenance testing.

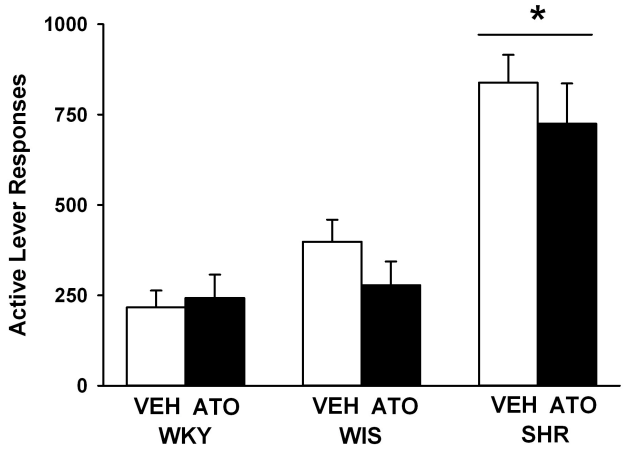

Figure 11. Active lever responding averaged across the maintenance testing baseline in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

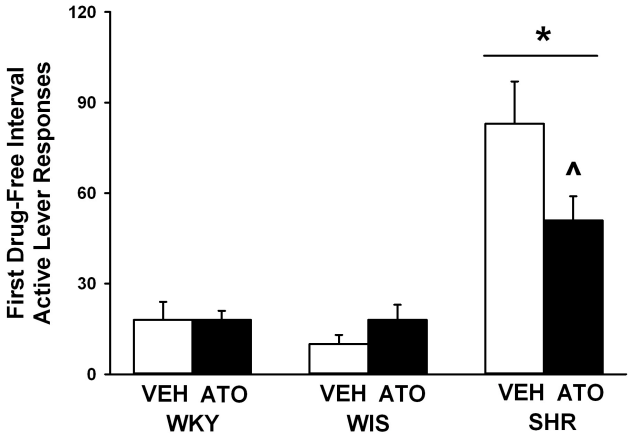

Figure 12. Active lever responding during the first drug-free interval of the final maintenance testing session in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Experiment 4b: Extinction Training. The number of sessions to reach the extinction criterion is shown in Figure 13. There were strain differences in number of sessions ($F(2, 42) = 6.1, p \leq 0.005$), with WKY and SHR requiring more sessions than WIS ($p \leq 0.04$ and $p \leq 0.005$ respectively). SHR and WKY did not differ. A strain X treatment interaction also was found ($F(2, 42) = 3.9, p \leq 0.02$). Post-hoc testing revealed that adult SHR receiving adolescent atomoxetine required more sessions to reach extinction criterion than SHR receiving vehicle ($p \leq 0.006$). Treatments did not differ in WKY and WIS. Analysis of the extinction baseline (Figure 14) revealed that the relative degree of extinguished responding (values expressed as percentage of the maintenance baseline) was not different between treatments and across strains. Inactive lever responses differed by strain ($F(2, 42) = 3.7, p \leq 0.03$), with SHR making more inactive lever responses (24 ± 5) than WIS ($11 \pm 3, p \leq 0.02$), but not WKY (15 ± 4).

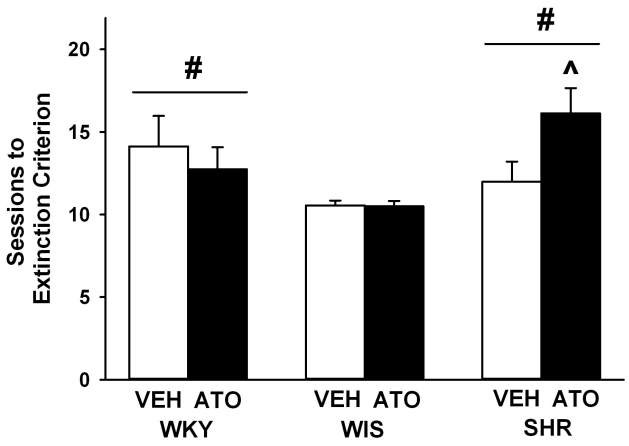

Figure 13. Number of sessions required to reach extinction criterion in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM). # $p \leq 0.05$ compared to WIS overall. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

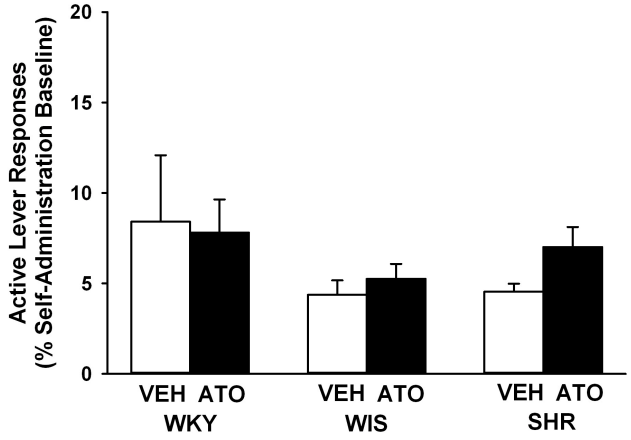

Figure 14. Active lever responding during the extinction baseline, expressed as a percentage of the maintenance testing baseline, in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence (Mean ± SEM).

Experiment 4c: Cue-Induced Reinstatement Testing. The number of active lever responses during reinstatement testing, averaged across the seven sessions of testing, and, for comparison, the first hour of the extinction baseline is shown in Figure 15. Three-factor ANOVA revealed main effects of phase ($F(1, 42) = 213.9$, $p \leq 0.001$) and strain ($F(2, 42) = 77.1$, $p \leq 0.001$), and a strain X treatment X phase interaction ($F(2, 42) = 3.1$, $p \leq 0.05$). Post-hoc testing of the interaction indicated that cue re-exposure during the reinstatement phase reinstated cocaine-seeking responses above extinction levels in each group ($p \leq 0.002$) and that adult SHR reinstated more cocaine-seeking responses than WKY or WIS ($p \leq 0.001$). In addition, SHR emitted more responses during the first hour of the extinction baseline than WKY or WIS ($p \leq 0.001$). During reinstatement testing, adolescent atomoxetine treatment attenuated cocaine-seeking responses compared to

vehicle only in adult SHR ($p \leq 0.032$). There were no strain or treatment differences in inactive lever responding.

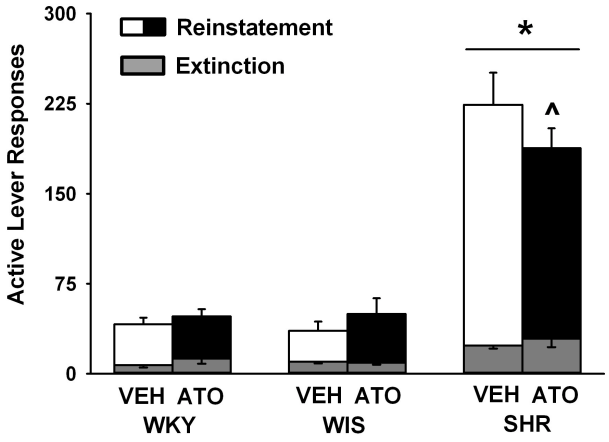

Figure 15. Active lever responding averaged across the seven reinstatement test sessions in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence. For comparison, active lever responses during the first hour of extinction training were averaged across the extinction baseline and are depicted in the gray bars (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Cocaine-seeking responses during reinstatement testing were additionally analyzed with reinstatement test session as a factor using a three-factor (strain X treatment X reinstatement session) ANOVA, with repeated measures for session. Active lever responses during the seven individual reinstatement testing sessions are shown in Figure 16. There was a main effect of strain ($F(2, 42) = 77.5$, $p \leq 0.001$). Further comparisons revealed that adult SHR overall made more active lever responses than WKY and WIS ($p \leq 0.001$). There were no significant effects of treatment, nor interactions between strain and treatment or reinstatement session in the three-way

ANOVA.

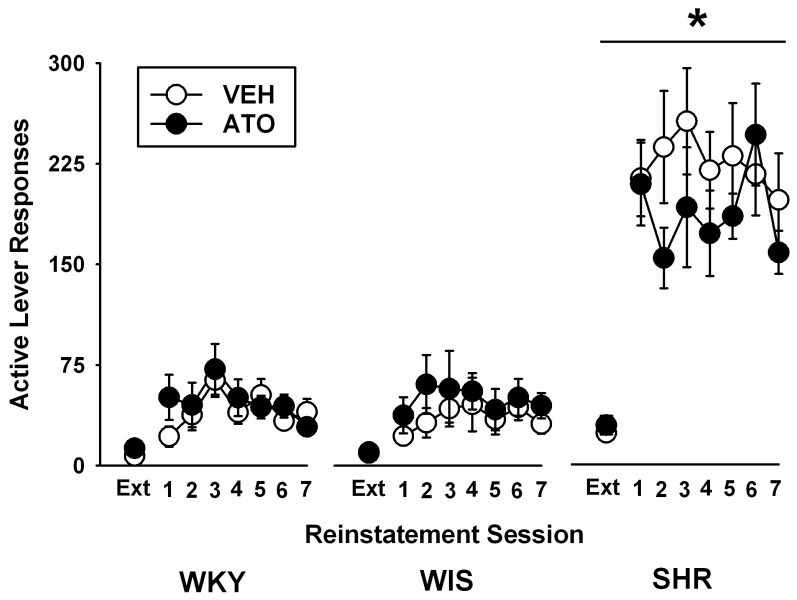

Figure 16. Active lever responses during each reinstatement test session in adult WKY, WIS, and SHR, following vehicle or atomoxetine treatment during adolescence. For comparison, active lever responses during the first hour of extinction (Ext) training, averaged across the extinction baseline, are also shown (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Discussion

Strain Differences in Cocaine Abuse Risk and Cocaine Cue Reactivity

The current work reproduces and extends previous research indicating that SHR are a valuable model of comorbid ADHD and cocaine abuse (Baskin et al, 2015; Harvey et al, 2011). As hypothesized, SHR exhibited elevated cocaine abuse risk compared to both WKY and WIS control strains, characterized by faster acquisition of cocaine self-

administration and increased efficacy and motivating influence of cocaine reinforcement. More specifically, during the acquisition phase adult SHR spontaneously acquired cocaine self-administration faster, earned more cocaine infusions, and made more cocaine-maintained responses than adult WKY and WIS. Importantly, acquisition of cocaine self-administration is dependent on dopamine D1 receptors in the nucleus accumbens shell, as silencing of D1a receptors in the shell region prevents acquisition of cocaine self-administration in outbred rats (Pisanu et al, 2015). While the effect of silencing D1 receptors in the accumbens shell in SHR have not been investigated, SHR exhibit elevated Fos expression in the nucleus accumbens core as well as the shell areas (Ohno et al, 2012). Fos-expressing neurons in nucleus accumbens are known to interact with D1 receptors, and thus have a critical role in cocaine reinforcement (Anderson et al, 2003; Bari & Pierce, 2005; Ikemoto et al, 1997). Together, these observations suggest that elevated activity at D1 receptors in the nucleus accumbens may contribute to faster acquisition of cocaine self-administration in SHR, relative to WKY or WIS control strains. Consistent with this view, D1 receptor expression in the nucleus accumbens is elevated in SHR compared to the WKY control strain (Ohno et al, 2012),

Under both FR and PR schedules, SHR continued to exhibit increased cocaine abuse risk, earning more cocaine infusions and making more cocaine-maintained responses across a range of doses, consistent with prior studies (Baskin et al, 2015; Harvey et al, 2011). Vulnerability to cocaine abuse is reflected by vertical (upward) shifts in FR and PR dose-response functions (Piazza et al, 2000). As with acquisition, motivation to self-administer cocaine under the PR schedule, in particular, is mediated by

D1 receptors, as infusion of D1 receptor antagonists in the nucleus accumbens, VTA, and mPFC reduces PR breakpoints for cocaine (McGregor & Roberts, 1993; 1995; Ranaldi & Wise, 2001). Interestingly, high-novelty seeking rats exhibit upward shifts in cocaine dose-response functions (Piazza et al, 2000). This behavior has been attributed to increased dopamine release in the nucleus accumbens following cocaine administration, as well as to elevated DAT function and D1 receptor expression in the nucleus accumbens (Hooks et al, 1991; 1994). Like individuals with ADHD, SHR exhibit high novelty seeking (dela Peña et al, 2015; Donfrancesco et al, 2015), as well as elevated striatal DAT density (Roessner et al, 2010) and increased D1 receptor expression (Ohno et al, 2012) in the nucleus accumbens, compared to WKY. These differences may contribute to the vertical shifts in cocaine dose-response functions observed in SHR.

Consistent with the hypotheses and with prior studies (Jordan et al, 2014), cocaine intake and cocaine seeking was also greater in SHR compared to WKY or WIS under the second-order schedule of reinforcement. High levels of cocaine seeking by SHR both when cocaine was (maintenance testing) and was not (the first drug-free interval of maintenance testing and reinstatement testing) available for self-administration suggest that SHR exhibit heightened cocaine cue reactivity compared to WKY or WIS. Notably, SHR extinguished active lever responding within the same timeframe as WKY, but more slowly than WIS. The observation that SHR exhibit similar rates of extinction compared to WKY suggests that extinction learning is not impaired in SHR, but rather may be faster in WIS. High levels of cocaine seeking by SHR during reinstatement testing are therefore not likely due to impaired extinction learning, but rather further reflect

heightened cocaine cue reactivity in this strain. SHR also often made more inactive lever responses than WKY and WIS, which may relate to hyperactivity in SHR when reinforcers are infrequent (Sagvolden et al, 2005). Nonetheless, SHR remained goal-directed, as active lever responding was substantially greater than inactive lever responding throughout maintenance and reinstatement testing.

Control strains did not differ in the various measures of cocaine abuse risk, with the exception that WIS earned more cocaine infusions and made more active lever responses than WKY for 0.1 mg/kg cocaine under the FR1 schedule. Importantly, the 0.1 mg/kg dose produced peak rates of responding in all strains under the FR1 schedule. Cocaine doses commonly abused in humans and those associated with peak rates of responding in non-human primates produce similar levels of striatal DAT occupancy (Wilcox et al, 2002), suggesting that strain differences in cocaine self-administration may reflect strain differences in DAT function or expression. Compared with WIS, WKY exhibit decreased DAT density in the nucleus accumbens (Jiao et al, 2003). The 0.1 mg/kg cocaine dose may therefore be a less efficacious reinforcer for WKY relative to WIS and SHR, due to lower DAT expression in reward-associated regions.

Effects of Adolescent Atomoxetine on Cocaine Abuse Risk and Cue Reactivity

Consistent with the hypotheses, adolescent treatment with a pharmacologically relevant dose of atomoxetine did not further enhance the speed to acquire cocaine self-administration, or the efficacy and motivating influence of cocaine reinforcement in adult SHR, in contrast to previous observations with methylphenidate. An exception was that

atomoxetine-treated SHR made more active lever responses at the acquisition criterion, without earning more cocaine infusions. These findings indicate that responding was greater during the 20-second timeout periods that followed each cocaine infusion and during which the cue light was illuminated. Acute atomoxetine treatment is known to have cognitive enhancing effects, via increased noradrenergic transmission (Arnsten & Pliszla, 2011; Gamo et al, 2010; Janak et al, 2012). Therefore, it is possible that prior adolescent atomoxetine treatment increased the salience of cocaine-related cues in SHR during acquisition when cocaine was first available for self-administration, but not later after chronic exposure to cocaine. Rather, adolescent atomoxetine modestly decreased cue reactivity in adult SHR when cocaine was not available, both during the first drug-free interval of maintenance testing and during cue-induced reinstatement, when responding was averaged across all seven reinstatement test sessions. Adolescent atomoxetine treatment also increased the number of sessions required to extinguish lever responding in adult SHR. However, all strains and treatment groups extinguished active lever responding to the same relative degree prior to reinstatement testing (i.e., ≤ 10% of the self-administration baseline). The slower rate of extinction in SHR did not result in elevated cocaine abuse risk, as assessed during subsequent reinstatement testing.

Adolescent atomoxetine treatment did not alter any measure of cocaine abuse risk in adult WIS, the outbred control strain. Although a previous study reported attenuated cocaine seeking during second-order maintenance testing in an outbred rat strain following acute administration of atomoxetine (Economidou et al, 2011), these effects were only observed with doses that were 3- to 10-fold higher (1 or 3 mg/kg) than the

more 0.3 mg/kg dose used in the present work, which may more closely resemble doses used in the treatment of ADHD (Bymaster et al, 2002). Interestingly, in contrast to its effects in SHR and WIS, adolescent atomoxetine treatment speeded acquisition of cocaine self-administration in adult WKY. Atomoxetine also differentially influenced strain performance on a strategy set shifting task, such that learning speed during the initial discrimination phase was faster in adolescent SHR, but slower in adolescent WKY and WIS, after atomoxetine treatment (Harvey et al, 2013). Taken together, these results emphasize the importance of concurrent evaluation of both inbred and outbred control strains when assessing the effects of adolescent ADHD medication in the SHR model.

In conclusion, these experiments further demonstrate the value of the SHR as a model for comorbid ADHD and cocaine abuse risk, and suggest that cocaine cue reactivity may contribute to the elevated risk of cocaine use disorder in individuals with ADHD. However, further studies evaluating physiological measures such as skin conductance, heart rate, or hemodynamic neural responses to drug cues in individuals with ADHD are necessary to confirm this hypothesis. Contrary to previous findings with methylphenidate, adolescent atomoxetine treatment did not further enhance cocaine abuse risk in SHR. Atomoxetine may therefore be a suitable alternative to methylphenidate treatment in teenagers with ADHD, in whom the risk of later drug abuse may be a concern. Moreover, while these experiments do not raise critical concerns about the safety of atomoxetine, they do emphasize the importance of accurate diagnosis of ADHD. In the WKY control strain, atomoxetine speeded acquisition of cocaine self-administration. Misdiagnosis of ADHD, and subsequent atomoxetine treatment in teens

could therefore result in a more rapid development of abuse of cocaine, if the drug is sampled.

CHAPTER THREE.

Aim 2: Effects of an Alternative Stimulant Medication on Prefrontal Cortex Function during Adolescence and Cocaine Abuse Risk and Cue Reactivity in Adulthood

Introduction

The experiments in Aim 1 tested the hypothesis that, in contrast to the stimulant methylphenidate, the non-stimulant ADHD medication, atomoxetine, does not increase cocaine abuse risk when treatment is initiated during adolescence. The findings suggest that adolescent treatment with atomoxetine does not increase cocaine abuse risk and may modestly decrease reactivity to cocaine-related cues. Despite these observations, atomoxetine conveys some disadvantages compared to methylphenidate. For example, atomoxetine often requires a week or longer to begin alleviating ADHD symptoms (Sibley et al, 2014). Moreover, as a non-stimulant, atomoxetine improves different symptoms of ADHD than methylphenidate, and may not be as clinically efficacious in some individuals (Cubillo et al, 2014; Faraone et al, 2006). Therefore, it is important to

identify an alternative stimulant drug, which satisfactorily improves ADHD symptoms but does not increase later cocaine abuse risk for teenagers with ADHD.

One potential alternative is d-amphetamine (Dexedrine®), a stimulant drug with a different mechanism of action than methylphenidate. Amphetamine is more efficacious than methylphenidate in relieving ADHD symptoms, and has a similar side effect profile (Arnold, 2000; Faraone & Buitelaar, 2010; Patrick & Morowitz, 1997; Sibley et al, 2014). Formulations of amphetamine often contain both d- and l-isomers. However, the l-isomer is not prescribed alone, and the d-isomer is more potent in mediating dopamine transmission and more efficacious in alleviating ADHD symptoms (Arnold et al, 1973; Heal et al 2012, 2013; Patrick & Markowitz 1997). Therefore, d-amphetamine is the focus of Aim 2.

Although both methylphenidate and d-amphetamine block DAT and NET, d-amphetamine also reverses activity at these transporters (Robertson et al, 2009). d-Amphetamine can additionally be taken up into the pre-synaptic terminal, where it reverses activity at vesicular monoamine transporter-2 (VMAT-2). Reversal of VMAT-2 prevents the sequestration and retention of monoamines within vesicles (Easton et al, 2007). Excess cytosolic monoamines subsequently accumulate, and are transported to the extracellular space through reversed activity at DAT and NET (Easton et al, 2007). Because of these additional mechanisms of action, d-amphetamine induces greater increases in extracellular monoamines than methylphenidate, even at therapeutically relevant doses (Kuczenski & Segal, 2001). Another mechanism of action exhibited by d-amphetamine, which is not shared with methylphenidate, is the ability to bind to the

intracellular trace amine-associated receptor 1 (TAAR1). TAAR1 is co-expressed with DAT in reward-associated regions, such as the substantia nigra, VTA, and amygdala (Lindemann et al, 2008; Xie et al, 2007). Activation of TAAR1 by amphetamine reduces cell surface expression of DAT, and may lead to decreased DAT function in reward-associated regions, such as the striatum (Miller, 2011; Xie & Miller, 2009). Thus, whereas adolescent methylphenidate treatment increases DAT function in mPFC of SHR and increases cocaine abuse risk (Somkuwar et al, 2013b), adolescent d-amphetamine treatment may reduce DAT expression and function. Adolescent d-amphetamine may therefore have long-term consequences for cocaine abuse risk that are different from methylphenidate.

In order to establish the SHR as a viable model for adolescent d-amphetamine treatment, it is first necessary to determine whether d-amphetamine improves ADHD-related deficits in SHR, as it does clinically. In adult SHR, d-amphetamine reduces hyperactivity and impulsivity, and improves sustained attention and short- and long-term memory (Meneses et al, 2011; Sagvolden & Xu, 2008). However, the effects of d-amphetamine in adolescent SHR are not well known. As discussed in Chapter One, the adolescent brain undergoes unique neurochemical changes, including altered expression of dopamine D1 receptors and DAT (Andersen, 2005; Brenhouse et al, 2008; Moll et al, 2000; Stanis & Andersen, 2014). These observations suggest that psychostimulants may differentially affect adolescents, relative to juveniles or adults. Experiment 5 therefore determines the effects of d-amphetamine treatment in adolescent WKY, WIS, and SHR, on PFC functioning using the strategy set shifting task (Floresco et al, 2008; Harvey et al,

2013). Previous studies using this task showed that adolescent SHR exhibit deficits in behavioral flexibility during the set shift phase, which is dependent on mPFC functioning (Birrell & Brown 2000; Floresco et al, 2008; Harvey et al, 2013; Ragozzino et al, 1999). During the set shift phase, atomoxetine improved performance in adolescent SHR (Harvey et al 2013). In contrast, methylphenidate improved performance in adolescent SHR during initial set formation (Harvey et al, 2013), a phase of the task that is dependent on catecholamine projections to PFC as a whole (Crofts et al, 2001). Neither drug in adolescent SHR improved reversal learning performance (Harvey et al, 2013), which is dependent on OFC functioning (Ghods-Sharifi et al, 2008). Interestingly, these findings are consistent with clinical observations that not all ADHD symptoms are sufficiently alleviated by single drug therapies (Wilens et al, 2009). Use of the strategy set shifting task in Experiment 5 therefore allows differentiation of the effects of d-amphetamine from those of methylphenidate and atomoxetine on PFC functioning in adolescent SHR.

If d-amphetamine improves ADHD-related cognitive deficits in adolescent SHR, it then becomes important to determine whether adolescent d-amphetamine treatment alters cocaine abuse risk in adulthood. Experiments 6 through 8 examined whether adolescent d-amphetamine treatment increases cocaine abuse risk in the SHR model. The speed to acquire cocaine self-administration, as well as the efficacy and motivating influence of cocaine reinforcement, were assessed in adult WKY, WIS, and SHR following discontinuation of adolescent treatment with a clinically relevant dose of d-amphetamine. Fixed-ratio (FR) and progressive ratio (PR) dose-response curves were

evaluated using multiple test doses of cocaine, in order to fully characterize cocaine's reinforcing effects and to be directly comparable to Aim 1.

Lastly, as reviewed in Chapter Two, another important aspect of cocaine abuse is reactivity to cocaine-related cues. The results of Experiment 4 show that SHR are more reactive to cocaine cues than control strains. Importantly, the environmental cues associated with cocaine use trigger neural activity and dopamine release in regions of the brain that are dysfunctional in SHR and in ADHD patients, including the mPFC and OFC (Ciccocioppo et al, 2001; Cubillo et al, 2011; Harvey et al, 2013; Jasinka et al, 2014; Nelissen et al, 2012; Wilcox et al, 2011). Repeated administration of a clinically relevant dose of amphetamine during early adolescence (P22 - 34) in outbred rats was shown to increase dendritic length, branching, and spine density in the mPFC (Heijtz et al, 2003). If adolescent d-amphetamine treatment induces long-term structural changes in PFC of SHR, it is possible that d-amphetamine may also modulate cocaine cue reactivity in this strain. Experiment 9 evaluated this possibility by examining cocaine cue reactivity and relapse behavior in adult SHR, WKY and WIS, following discontinuation of adolescent d-amphetamine treatment. To be directly comparable to Aim 1, a second-order schedule of cocaine delivery and cue presentation was used. Cocaine-seeking behavior was therefore measured during maintenance testing (cocaine available for self-administration) and reinstatement testing (cocaine not available for self-administration).

Experiment 5: Effects of d-Amphetamine on Strategy Set Shifting in Adolescent Rats

Experiment 5 tested the hypothesis that adolescent SHR exhibit deficits in behavioral flexibility during the strategy set shifting task (e.g., requiring more trials and time to reach learning criterion) compared to adolescent WKY and WIS control strains. Experiment 5 also tested the hypothesis that adolescent SHR treated with a clinically relevant dose of d-amphetamine show improved performance during one or more phases of the strategy set shifting task, compared to vehicle-treated SHR.

Materials and Methods

Animals and Treatments. All procedures were approved by the Institutional Animal Care and Use Committee at Boston University, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Eighth Edition). Male WKY/Cr, WIS/Cr, and SHR/Cr rats (Charles River Laboratories, Wilmington, MA, Kingston, NY or Raleigh, NC) arrived on P25. Group sizes for Experiment 5 were $n = 9$ for vehicle and d-amphetamine-treated WKY and WIS, $n = 10$ for vehicle-treated SHR, and $n = 12$ for d-amphetamine-treated SHR. See Figure 17 for an experimental timeline.

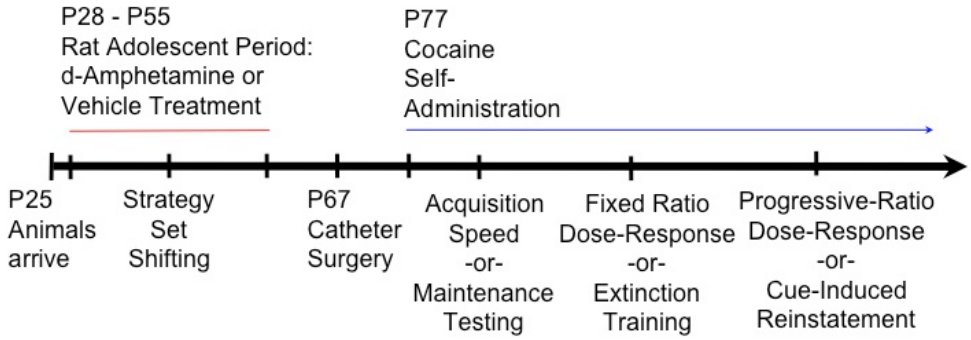

Figure 17. Timeline of experimental events in Aim 2.

Animal housing conditions were identical to those described in Experiment 1. From P28 through P55, constituting the rat adolescent period (Spear, 2000), animals received once daily i.p. injections of either a clinically relevant dose of d-amphetamine (0.5 mg/kg, Sigma-Aldrich, St. Louis, MO) dissolved in 0.9% sterile saline, or saline alone (2 ml/kg). The double injection volume was used to be consistent with Experiments 1 through 4. Treatments were administered once daily, Monday-Friday. The selected dose of d-amphetamine produces plasma drug concentrations within the clinical range and is below the threshold for causing locomotor activation (Brown et al, 1979; Heitjz et al, 2003; Labonte et al, 2011). The chosen dose of d-amphetamine also increases dopamine and norepinephrine activity and induces long-term structural changes in the PFC (Heijtz et al, 2003; Kuczenski & Segal, 2001; Labonte et al, 2011). Treatments were administered 30 minutes prior to behavioral testing in the strategy set shifting task, to allow peak monoamine responses to occur and to ensure that plasma levels reached clinical significance during testing (Heijtz et al, 2003; Kuczenski & Segal, 2001). Food

was restricted from P28-P55 to ~90% of a growth-adjusted free-feeding body weight, to facilitate lever responding for food reinforcement during the set shifting task, and to mimic conditions of past comparator studies (Harvey et al, 2011; 2013; Somkuwar et al 2013a, b). Animals had free access to water throughout the experiment, and food was freely available after P55.

Testing Environment. Experimental chambers were identical to those described in Experiment 1, except that response levers were retractable (as in Harvey et al, 2013). Pellet dispensers for food delivery were also located inside the cubicle enclosing each operant chamber (Med Associates).

Habituation Training. An operant version of the strategy set shifting task was used (Floresco et al, 2008; Harvey et al, 2013). On P28, all animals received 20 chocolate-flavored pellets in their home cages, to habituate rats to the novel food. On P31, all rats began training in experimental chambers. Rats were required to lever press under a FR1 schedule to earn a minimum of 25 chocolate pellet reinforcers within a 30-min period. Thereafter, rats began lever retraction training, requiring a response within 10-sec of lever insertion into the chamber to earn a chocolate pellet reinforcer. Once rats made fewer than 5 omissions for four consecutive 45-min daily sessions, lever position bias was established by allowing rats to freely press either lever for ~10-min. The lever on which the most responses were made was defined as preferred.

Experiment 5a: Initial Set Formation. On P42, rats were required to adopt a visual-based strategy to earn reinforcement. All set shift test sessions were 2 hours in length, and were conducted during the light phase once daily, Monday - Friday, at approximately the same time each day. During the initial set formation, each trial began with a 20-sec timeout in a dark chamber. After 20 sec, one of the two stimulus lights was illuminated (randomly selected for each trial). The house light illuminated 3-sec later, and both response levers were inserted into the chamber. Rats were required to press the lever under the illuminated stimulus light, regardless of lever bias, to earn a chocolate pellet reinforcer (see Figure 18). Levers were retracted after a response was made (whether correct or incorrect), or if 10-sec elapsed with no response (omission). After a correct response, the stimulus light remained illuminated for an additional 4 sec, and a chocolate pellet was delivered 15 sec later. The house light remained illuminated until 4 sec after pellet delivery. A 15-sec delay to reinforcement was imposed to increase cognitive demand and to better detect strain and treatment differences, as SHR are more sensitive to delayed reinforcers than WKY (Harvey et al, 2013; Johansen et al, 2005). After an incorrect response or a trial omission, the stimulus and house lights were immediately extinguished and a new trial was initiated. Training on the initial set formation continued until criterion was reached, defined as making 8 consecutive correct responses, following Floresco et al, 2008, and Harvey et al, 2013.

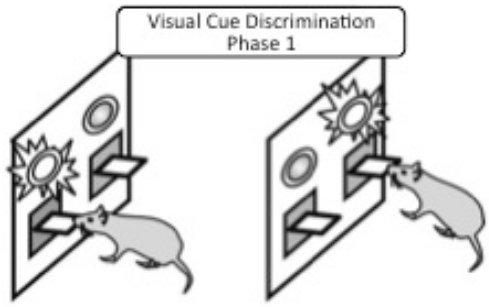

Figure 18. Initial set formation requires pressing the lever under the illuminated cue light (visual cue discrimination). Adapted from Floresco et al, 2008.

Experiment 5b: Set Shift. On the day following completion of the initial set formation, rats were required to shift responding to a spatial-based discrimination strategy. During this phase, animals were required to press the lever opposite the lever position bias to earn reinforcement, regardless of which stimulus light was illuminated (see Figure 19). Trial contingencies were otherwise identical to the initial set formation. Training on the set shift phase continued until rats made 10 consecutive correct responses (Floresco et al, 2008; Harvey et al, 2013).

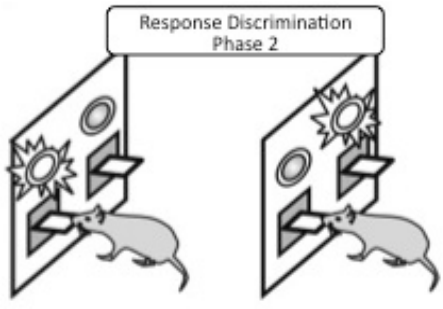

Figure 19. The set shift requires pressing the lever opposite from the preferred lever (response discrimination). Adapted from Floresco et al, 2008.

Experiment 5c: Reversal Learning. On the day following completion of the set shift phase, reversal learning was assessed. During this final phase of the task, rats were required to press the lever on the same side as the lever position bias, regardless of which stimulus light was illuminated (see Figure 20). Trial contingencies were otherwise identical to those of the initial set and set shift phases. Training on reversal learning continued until rats made 10 consecutive correct responses (Floresco et al, 2008; Harvey et al, 2013).

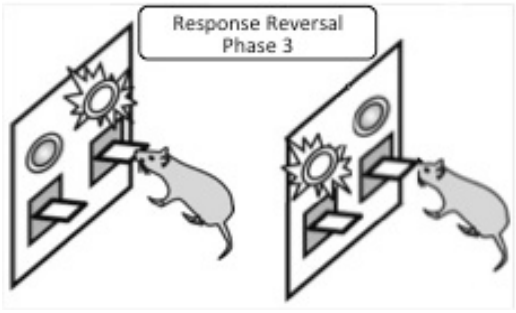

Figure 20. Reversal learning requires pressing the preferred lever (response reversal). Adapted from Floresco et al, 2008.

Data Analysis. Dependent measures for the strategy set shifting task included number of trials completed to reach criterion, latency to reach criterion (included the time for omitted trials), number of omitted trials, average lever press reaction time, variability in lever press reaction time, and ratio of correct to incorrect lever responses (choice accuracy), until criterion was reached. In addition, error subtypes were coded for the set shift and reversal learning phases of the task (Harvey et al, 2013; Floresco et al, 2008). During the set shift phase, perseverative and regressive errors were recorded when rats

pressed a lever with the stimulus light illuminated above it on trials that required pressing of the opposite lever. Errors were perseverative when rats pressed the incorrect lever on six or more trials per block of eight trials. Once rats made five or fewer incorrect choices in a block of eight trials for the first time, the incorrect lever choices in subsequent blocks were scored as regressive errors. Never-reinforced errors were recorded when a rat pressed the incorrect lever on trials when the correct lever had the stimulus light illuminated above it (i.e., a choice that was not reinforced during either the initial set or set shift phase). During the reversal-learning phase, errors were examined in blocks of 16 trials and consisted of perseverative and regressive errors (Ghods-Sharifi et al, 2008; Harvey et al, 2013). Once rats made ten or fewer incorrect choices in a block of 16 trials for the first time, the incorrect lever choices in subsequent blocks were then scored as regressive errors. Never-reinforced errors are not possible during the reversal-learning phase. Perseverative errors are an index of how well the previously acquired strategy is suppressed, regressive errors are an index of how well the new strategy is maintained, and never-reinforced errors are an index of how well the new strategy is acquired (Floresco et al, 2008). Measures of the strategy set shifting task were square root transformed prior to analysis due to non-normality in the majority of measures. Measures were analyzed by two-factor (strain X treatment) ANOVAs, followed by post-hoc Tukey tests.

Results

Experiment 5a: Initial Set Formation. Figure 21 depicts data obtained during the initial set formation phase of the strategy set shifting task. Strains differed in trials to criterion ($F(2, 52) = 5.4, p \leq 0.008$), latency to criterion ($F(2, 52) = 5.3, p \leq 0.008$), trial omissions ($F(2, 52) = 4.5, p \leq 0.01$), average reaction time ($F(2, 52) = 9.2, p \leq 0.001$), and reaction time variability ($F(2, 52) = 9.3, p \leq 0.001$). No strain differences were observed for choice accuracy. Adolescent SHR and the WIS control required fewer trials to reach criterion ($p \leq 0.01$ and 0.02, respectively) and had shorter latencies to reach criterion ($p \leq 0.01$ and 0.03, respectively) than the WKY control. Both SHR and the WKY control exhibited slower reaction time and greater reaction time variability than the WIS control ($p \leq 0.01$). The WKY control also made more trial omissions than the WIS control ($p \leq 0.01$). d-Amphetamine treatment did not alter performance on any measure across strains during initial set formation.

Experiment 5b: Set Shift. Figure 22 depicts data obtained during the set shift phase of the strategy set shifting task. Strains differed in trials to criterion ($F(2, 52) = 4.8, p \leq 0.01$), latency to criterion ($F(2, 52) = 6.4, p \leq 0.003$), trial omissions ($F(2, 52) = 3.7, p \leq 0.03$), average reaction time ($F(2, 52) = 3.7, p \leq 0.03$), and reaction time variability ($F(2, 52) = 8.7, p \leq 0.001$). No strain differences were observed for choice accuracy. Adolescent SHR and the WIS control required more trials to reach criterion (ps ≤ 0.02) and had longer latencies to reach criterion ($p \leq 0.009$ and 0.008, respectively) than the WKY control. Adolescent SHR also exhibited greater reaction time variability

compared to the WKY and WIS controls ($p \leq 0.001$ and 0.04, respectively). Moreover, SHR exhibited slower reaction times ($p \leq 0.02$) and made more omissions ($p \leq 0.03$) than the WKY control.

For latency to criterion, there was a trend towards a strain X treatment interaction ($F(2, 52) = 2.7, p \leq 0.07$) and further testing revealed that vehicle-treated WKY took less time to reach criterion than vehicle-treated WIS and SHR ($p \leq 0.01$ and 0.001, respectively). d-Amphetamine treatment reduced latency to criterion in adolescent SHR only ($p \leq 0.01$). There was also a trend towards a strain X treatment interaction ($F(2, 52) = 2.7, p \leq 0.07$) for choice accuracy, which improved relative to vehicle following d-amphetamine treatment in adolescent SHR ($p \leq 0.01$).

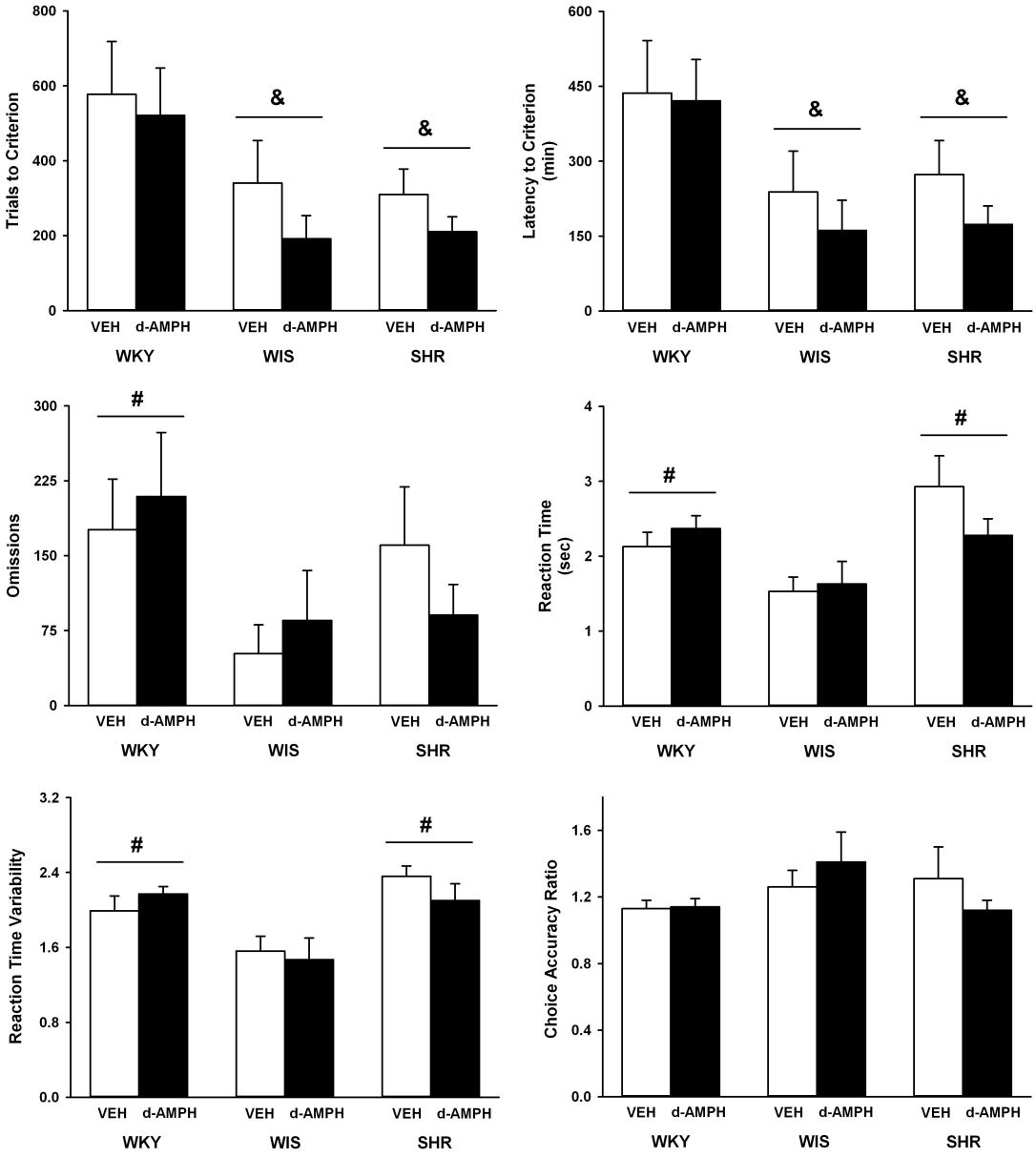

Figure 21. Trials to criterion, latency to criterion, trial omissions, lever press reaction time, variability in reaction time, and choice accuracy during initial set formation in adolescent WKY, WIS, and SHR following vehicle or d-amphetamine treatment (Mean ± SEM). & $p \leq 0.05$ compared to WKY overall. # $p \leq 0.05$ compared to WIS overall.

Moreover, although the strain X treatment interaction was not significant, further testing revealed that d-amphetamine treatment reduced the number of trials required to reach criterion in SHR compared to vehicle treatment ($p \leq 0.04$).

With respect to regressive errors, there was a significant strain X treatment interaction ($F(2, 52) = 3.3$, $p \leq 0.04$). Vehicle-treated SHR made more regressive errors than vehicle-treated WKY ($p \leq 0.01$). Compared to vehicle treatment, d-amphetamine reduced regressive errors in SHR only ($p \leq 0.006$). For never-reinforced errors, both strain ($F(2, 52) = 4.6$, $p \leq 0.01$) and treatment ($F(1, 52) = 3.9$, $p \leq 0.05$) effects were observed. Overall, the WIS control made more never-reinforced errors than the WKY control ($p \leq 0.01$). In addition, rats treated with d-amphetamine made fewer never-reinforced errors than vehicle-treated rats overall ($p \leq 0.05$). Although the strain X treatment interaction was not significant, further testing revealed that SHR treated with d-amphetamine made fewer never-reinforced errors than vehicle-treated SHR ($p \leq 0.04$). No strain or treatment differences were observed for perseverative errors during the set shift phase.

Experiment 5c: Reversal Learning. Figure 23 depicts data obtained during the reversal learning phase of the strategy set shifting task. Strain differences were observed in trials to criterion ($F(2, 52) = 3.8$, $p \leq 0.03$), latency to criterion ($F(2, 52) = 5.1$, $p \leq 0.01$), trial omissions ($F(2, 52) = 4.7$, $p \leq 0.01$), average reaction time ($F(2, 52) = 5.6$, $p \leq 0.006$), and reaction time variability ($F(2, 52) = 6.4$, $p \leq 0.003$). No strain differences were observed for choice accuracy.

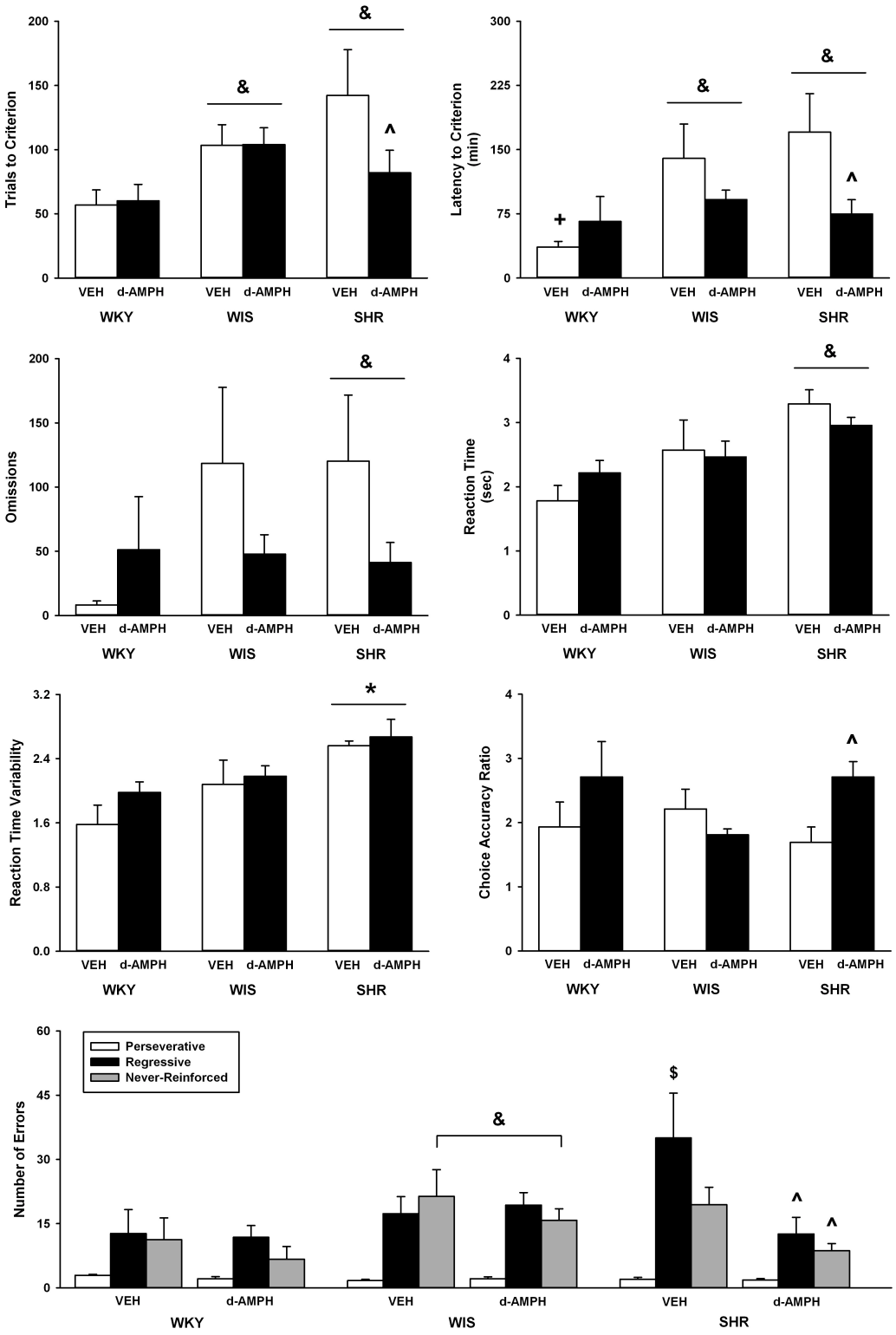
Trials to Criterion
Latency to Criterion (min)
Omissions
Reaction Time (sec)
Reaction Time Variability
Choice Accuracy Ratio
Number of Errors
Perseverative
Regressive
Never-Reinforced
VEH
d-AMPH
WKY
WIS
SHR

Figure 22. Trials to criterion, latency to criterion, trial omissions, lever press reaction time, variability in reaction time, choice accuracy, and error subtypes during the set shift phase in adolescent WKY, WIS, and SHR following vehicle or d-amphetamine treatment (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. & $p \leq 0.05$ compared to WKY overall. # $p \leq 0.05$ compared to WIS overall. + $p \leq 0.05$ compared to vehicle treatment in both other strains. $ $p \leq 0.05$ compared to vehicle-treated WKY. ^ $p \leq 0.05$ compared to vehicle-treated SHR.

Overall, adolescent SHR required fewer trials to reach the reversal learning criterion than the WIS control ($p \leq 0.02$). Both the WKY control and SHR had shorter latencies to reach criterion than the WIS control ($p \leq 0.01$ and 0.03, respectively). Adolescent WKY also made fewer trial omissions than the WIS control ($p \leq 0.01$). SHR exhibited slower and more variable reaction times than the WKY control ($p \leq 0.005$ and 0.003, respectively).

For perseverative errors, there was a trend towards a main effect of strain ($F(2, 52) = 2.9$, $p \leq 0.06$). Further testing revealed that SHR made fewer perseverative errors than the WIS control ($p \leq 0.05$). No strain differences were observed for regressive errors. d-Amphetamine treatment did not alter performance on any measure across strains during the reversal learning phase.

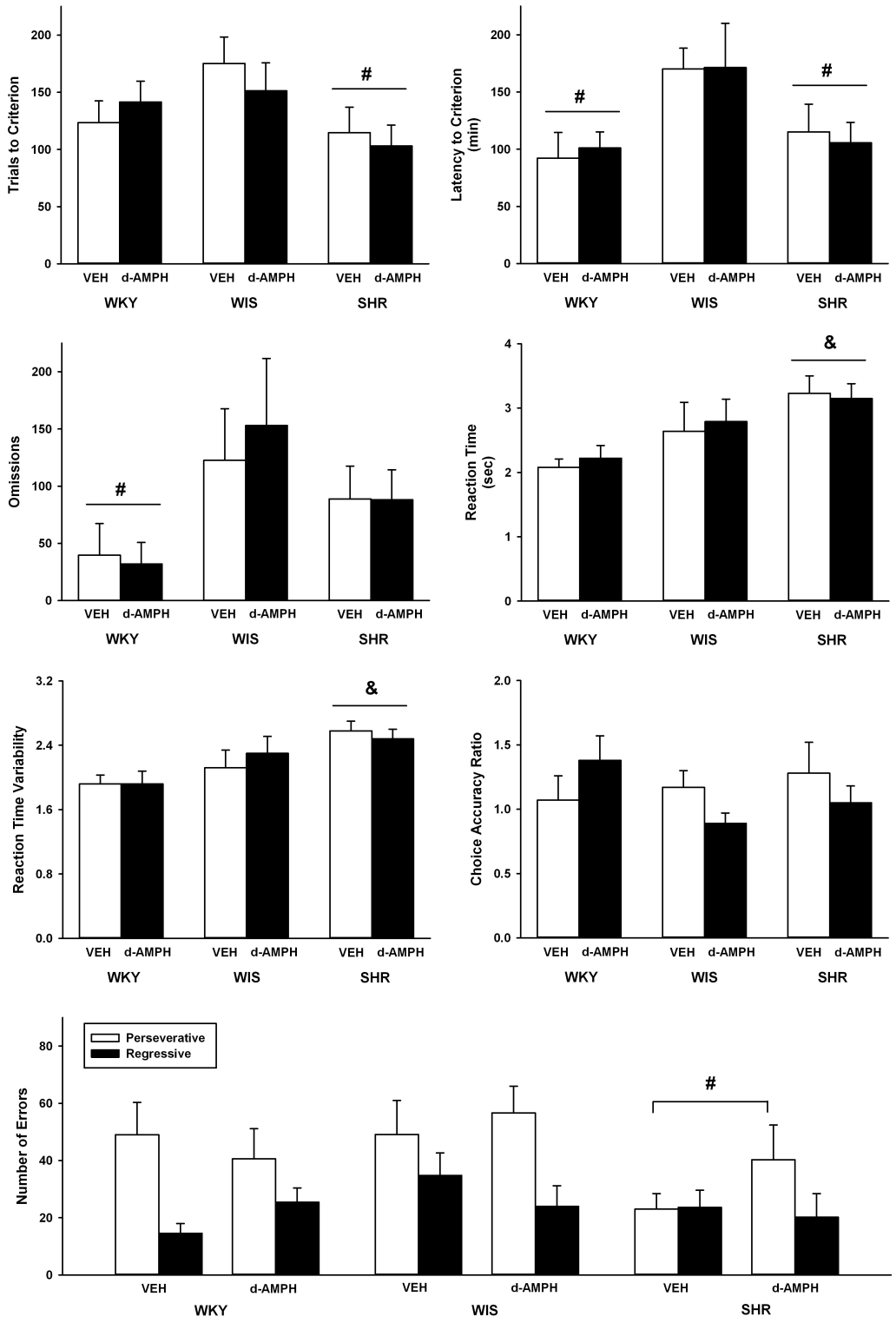
Trials to Criterion
Latency to Criterion (min)
Omissions
Reaction Time (sec)
Reaction Time Variability
Choice Accuracy Ratio
Number of Errors
Perseverative
Regressive
VEH
d-AMPH
WKY
WIS
SHR
#
&

Figure 23. Trials to criterion, latency to criterion, trial omissions, lever press reaction time, variability in reaction time, choice accuracy, and error subtypes during the reversal learning phase in adolescent WKY, WIS, and SHR following vehicle or d-amphetamine treatment (Mean ± SEM). & $p \leq$ compared to WKY overall. # $p \leq 0.05$ compared to WIS overall.

Experiment 6: Acquisition of Cocaine Self-Administration Following Adolescent d-Amphetamine Treatment

Experiment 6 sought to reproduce findings from Experiment 1, showing that adult SHR acquire cocaine self-administration faster, earn more cocaine infusions and make more lever responses compared to WKY and WIS control strains. Experiment 6 also tested the hypothesis that, in contrast to methylphenidate, treatment with a clinically relevant dose of d-amphetamine during adolescence does not increase the speed to acquire cocaine self-administration in adult SHR after adolescent treatment is discontinued.

Materials and Methods

New groups of SHR, WKY, and WIS were used in Experiment 6. Rats were treated with a clinically relevant dose of d-amphetamine, or saline alone, during adolescence, as described in Experiment 5. Group sizes for Experiment 6 were $n = 8$ per strain and treatment group.

Surgical procedures, testing environments, cocaine delivery and cocaine self-administration procedures were identical to those detailed in Experiment 1. Briefly, after

recovery from surgical implantation of i.v. catheters, animals were allowed to spontaneously acquire cocaine self-administration under a FR1 schedule of reinforcement. Training continued until acquisition criterion was reached, defined as earning ≥ 20 infusions for two consecutive sessions, and discriminating the active from inactive lever by a factor of 2 or greater. All rats reached acquisition criterion in 25 sessions or less.

Data Analysis. Dependent measures in Experiment 6 included the number of sessions required to reach acquisition criterion (square root transformed prior to analysis due to non-normality), as well as cocaine infusions earned and active and inactive lever responses at acquisition criterion. Measures were analyzed by two-factor (strain X treatment) ANOVAs followed by post-hoc Tukey tests.

Results

The number of sessions required to reach acquisition criterion are shown in Figure 24. Strains differed significantly ($F(2, 42) = 14.7, p \leq 0.001$), and there was also a strain X treatment interaction ($F(2, 42) = 6.6, p \leq 0.003$). Vehicle and d-amphetamine-treated SHR acquired cocaine self-administration faster than vehicle- and d-amphetamine-treated WKY and WIS (ps ≤ 0.03). Adolescent d-amphetamine slowed acquisition in adult WIS ($p \leq 0.004$), but speeded acquisition in adult WKY ($p \leq 0.04$). d-Amphetamine did not alter acquisition speed in SHR.

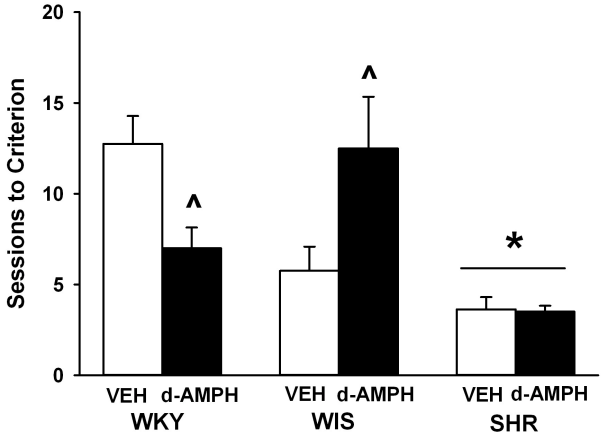

Figure 24. Number of sessions required to reach acquisition criterion in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

The number of cocaine infusions earned at the acquisition criterion is shown in Figure 25. Differences in strain ($F(2, 42) = 16.4$, $p \leq 0.001$) and treatment ($F(2, 42) = 9.1$, $p \leq 0.004$) were observed, as well as a trend towards a strain X treatment interaction ($F(2, 42) = 2.7$, $p \leq 0.08$). Overall, SHR earned more cocaine infusions than WKY and WIS ($p \leq 0.001$ and 0.03, respectively). WIS also earned more infusions than WKY ($p \leq 0.008$). Moreover, d-amphetamine-treated rats earned fewer infusions than vehicle-treated rats ($p \leq 0.005$). This effect was driven primarily by reduced cocaine intake in d-amphetamine-treated WIS and SHR compared to their vehicle counterparts ($p \leq 0.008$ and 0.01, respectively). Adolescent d-amphetamine did not alter cocaine intake in adult WKY.

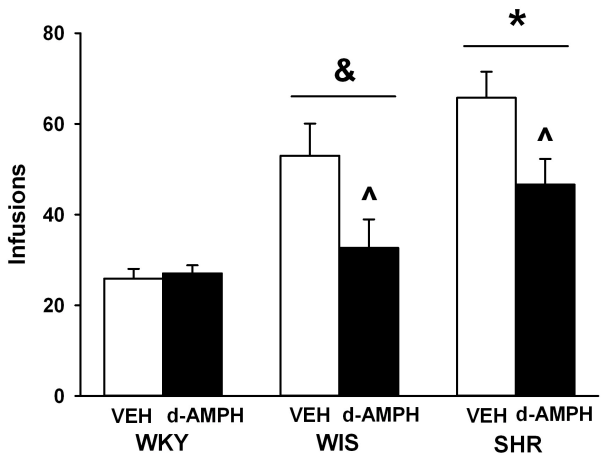

Figure 25. Number of cocaine infusions earned at acquisition criterion in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. & $p \leq 0.05$ compared to WKY overall. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Active lever responses at the acquisition criterion are shown in Figure 26. Strains ($F(2, 42) = 10.5, p \leq 0.001$) and treatments ($F(1, 42) = 6.2, p \leq 0.01$) again differed. There was also a trend towards a strain X treatment interaction ($F(2, 42) = 2.6, p \leq 0.08$). Overall, SHR made more active lever responses than WKY and WIS ($p \leq 0.001$ and 0.01, respectively). d-Amphetamine-treated rats made fewer active lever responses than VEH-treated rats ($p \leq 0.01$). This effect was driven primarily by reduced active lever responding in d-amphetamine-treated SHR relative to vehicle-treated SHR ($p \leq 0.004$). Adolescent d-amphetamine did not significantly alter active lever responding at acquisition in adult WKY or WIS. There was also a strain difference in inactive lever responding at criterion ($F(2, 42) = 3.8, p \leq 0.03$), with SHR making made more inactive lever responses (10.4 ± 3.4) than WKY (3.1 ± 0.6; $p \leq 0.05$). However, all strains

discriminated the active from the inactive lever by a factor of 2 or greater (10:1 for WKY, 16:1 for WIS, and 9:1 for SHR, on average).

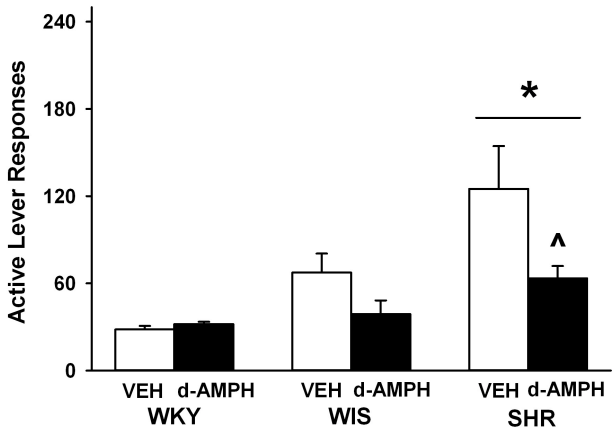

Figure 26. Number of active lever responses made at acquisition criterion in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Experiment 7: Fixed-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent d-Amphetamine Treatment

Experiment 7 sought to reproduce findings in Experiment 2, showing that the efficacy of cocaine reinforcement is elevated in adult SHR compared to WKY or WIS, such that SHR earn more cocaine infusions and make active lever responses across a range of doses under a FR1 schedule of reinforcement. Experiment 7 also tested the hypothesis that, in contrast to the effects of methylphenidate, treatment with a clinically

relevant dose of d-amphetamine during adolescence does not increase the efficacy of cocaine reinforcement in adult SHR after adolescent treatment is discontinued.

Materials and Methods

Cocaine self-administration procedures in Experiment 7 were identical to those detailed in Experiment 2. Briefly, following achievement of acquisition criterion in Experiment 6, a stable baseline level of responding for the 0.3 mg/kg training dose was established in all animals. A range of cocaine doses (0.003 to 1.0 mg/kg/infusion) was then substituted in pseudo-random order, every Tuesday and Friday. The 0.3 mg/kg training dose was available on intervening days, and for 2-3 days following completion of full FR1 cocaine dose-response functions. One vehicle- and one d-amphetamine-treated SHR expired prior to completing Experiment 7, and were therefore excluded from Experiment 7 data analyses.

Data Analysis. Dependent measures for Experiment 7 included the number of cocaine infusions earned, and active and inactive lever responses for each cocaine dose. Measures were analyzed by three-factor (strain X treatment X dose) ANOVAs, with repeated measures for dose followed by post-hoc Tukey tests.

Results

Cocaine dose-response functions based on the number of infusions earned under the FR1 schedule are shown in Figure 27. Three-way ANOVA identified differences in

strain (F (2, 40) = 36.3, $p \leq 0.001$) and dose (F (5, 200) = 87, $p \leq 0.001$). There were also interactions for strain X treatment (F (2, 40) = 3.9, $p \leq 0.03$), strain X dose (F (10, 200) = 8, $p \leq 0.001$), and strain X treatment X dose (F (10, 200) = 2.5, $p \leq 0.007$). Overall, SHR earned more infusions than WKY and WIS at 0.003, 0.01, 0.03, and 0.1 mg/kg cocaine (ps ≤ 0.001), and more infusions than WKY at 0.3 mg/kg cocaine ($p \leq 0.006$). WIS earned more infusions than WKY at 0.1 mg/kg cocaine ($p \leq 0.004$). Adolescent d-amphetamine reduced intake at 0.1 and 0.3 mg/kg cocaine in adult WIS ($p \leq 0.001$ and 0.01, respectively), and did not alter intake in adult WKY or SHR.

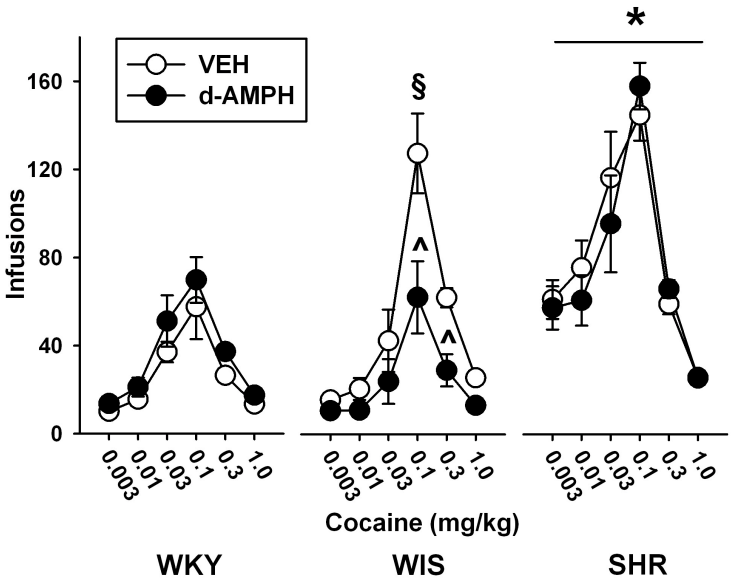

Figure 27. Cocaine dose-response functions based on infusions earned under a FR1 schedule of reinforcement by adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. § $p \leq 0.05$ compared, overall, to the same dose in WKY. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Analyses of the cocaine dose-response functions based on the number of active lever responses (Figure 28) were similar to the infusions analysis. For the three-way ANOVA, differences in strain ($F(2, 40) = 30.1$, $p \leq 0.001$) and dose ($F(5, 200) = 50.3$, $p \leq 0.001$) were observed. There was also a strain X dose interaction ($F(10, 200) = 7.1$, $p \leq 0.001$), and a trend towards a strain X treatment interaction ($F(2, 40) = 2.8$, $p \leq 0.07$). SHR made more active lever responses than WKY and WIS for 0.003, 0.01, 0.03, and 0.1 mg/kg cocaine (ps ≤ 0.001). Adolescent d-amphetamine treatment reduced active lever responses for 0.1 and 0.3 mg/kg cocaine in adult WIS ($p \leq 0.001$ and 0.05, respectively), and did not alter active responses in adult WKY or SHR. Analysis of inactive responding during FR1 dose-response testing also revealed a strain difference ($F(2, 40) = 4.3$, $p \leq 0.02$). SHR made more inactive responses (59.1 ± 16.7) than WKY (9.6 ± 1; $p \leq 0.02$).

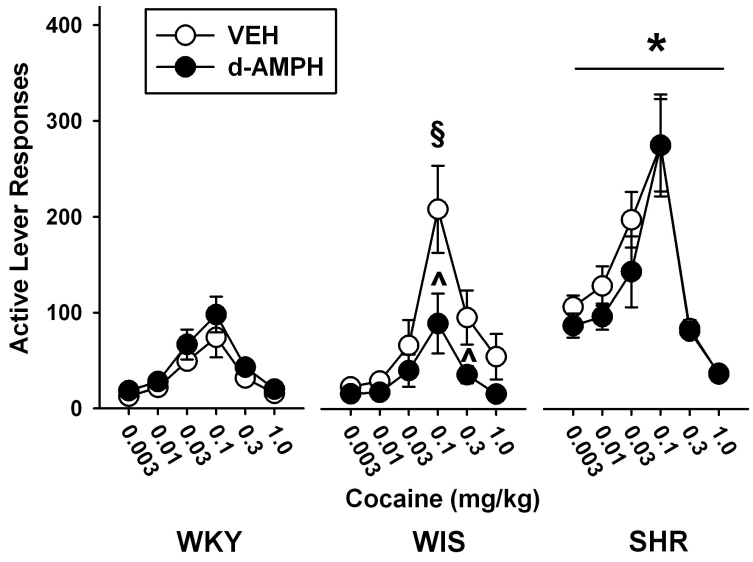

Figure 28. Cocaine dose-response functions based on active lever responses made under a FR1 schedule of reinforcement by adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. § $p \leq 0.05$ compared, overall, to the same dose in WKY. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Experiment 8: Progressive-Ratio Cocaine Dose-Response Functions in Adulthood Following Adolescent d-Amphetamine Treatment

Experiment 8 sought to reproduce findings of Experiment 3, showing that the motivating influence of cocaine reinforcement is elevated in adult SHR compared to WKY or WIS, such that SHR work harder to earn cocaine reinforcement, earn more cocaine infusions and make more active lever responses across a range of doses under a PR schedule. Experiment 8 also tested the hypothesis that, in contrast to the effects of methylphenidate, treatment with a clinically relevant dose of d-amphetamine during adolescence does not increase the efficacy of cocaine reinforcement in adult SHR after adolescent treatment is discontinued.

Materials and Methods

Cocaine self-administration procedures in Experiment 8 were identical to those detailed in Experiment 3. Briefly, after rats returned to baseline levels of responding for 0.3 mg/kg cocaine on the FR1 schedule in Experiment 7, a PR schedule of reinforcement was instituted. This schedule required rats to press the active lever an incrementally greater number of times to earn each subsequent cocaine infusion. In Experiment 8, self-administration sessions were terminated when rats failed to reach the next response requirement within 1 hour or after 4.5 hours had elapsed, which ever occurred first. After stable levels of responding for 0.3 mg/kg were established under the PR schedule,

cocaine test doses (0.01 - 1.0 mg/kg) were substituted in descending order, with 2-3 consecutive sessions allocated to each dose. Baseline responding was reestablished for 0.3 mg/kg cocaine after the full PR dose-response function was completed. One vehicle- and one d-amphetamine-treated WKY and an additional d-amphetamine-treated SHR expired prior to completing Experiment 8, and were thus excluded from Experiment 8 data analyses. At the end of Experiment 8, animals were humanely euthanized with an overdose of sodium pentobarbital (Euthasol).

Data Analysis. Dependent measures for Experiment 8 included PR breakpoints, the number of cocaine infusions earned, and active and inactive lever responses for each cocaine dose. Measures were analyzed by three-factor (strain X treatment X dose) ANOVAs, with repeated measures for dose, followed by post-hoc Tukey tests.

Results

Cocaine dose-response functions based on the PR breakpoint are shown in Figure 29. Three-way ANOVA identified differences in strain ($F(2, 37) = 42.6, p \leq 0.001$) and dose ($F(3, 111) = 71.6, p \leq 0.001$). There was also a significant strain X dose interaction ($F(6, 111) = 6.5, p \leq 0.001$) and a trend towards a treatment difference ($F(1, 37) = 3.2, p \leq 0.08$). SHR reached higher breakpoints than WKY and WIS across all cocaine doses (ps ≤ 0.001, except for 0.01 mg/kg where ps ≤ 0.05). Adolescent d-amphetamine treatment reduced PR breakpoints in adult WIS at 0.3 and 1.0 mg/kg cocaine ($p \leq 0.05$

and 0.001, respectively), and did not significantly alter PR breakpoints in adult WKY or SHR.

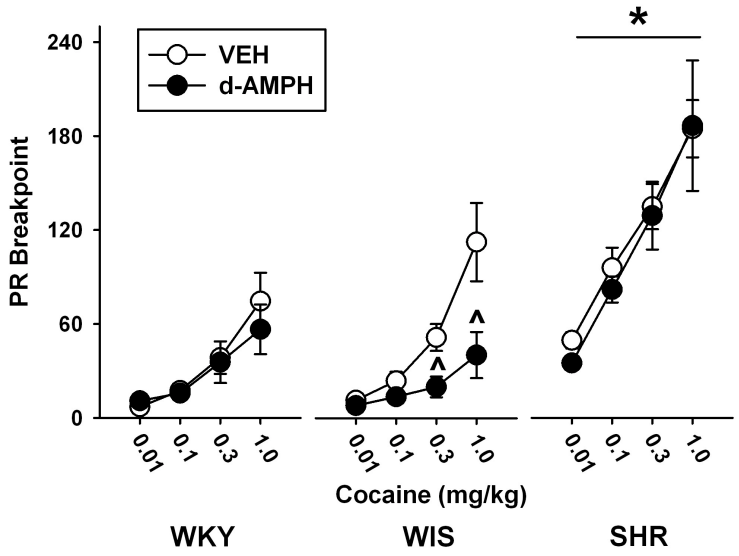

Figure 29. Cocaine dose-response functions, based on breakpoints reached under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

The PR cocaine dose-response functions based on the number of cocaine infusions earned are shown in Figure 30. Three-way ANOVA revealed differences in strain ($F(2, 37) = 43.6$, $p \leq 0.001$), treatment ($F(1, 37) = 5.8$, $p \leq 0.02$), and dose ($F(3, 111) = 120.4$, $p \leq 0.001$). There was also a treatment X dose ($F(3, 111) = 3.4$, $p \leq 0.02$) and strain X treatment X dose ($F(6, 111) = 2.2$, $p \leq 0.05$) interaction, and a trend towards a strain X treatment interaction ($F(2, 37) = 2.8$, $p \leq 0.07$). Overall, SHR earned more cocaine infusions than WKY and WIS across all cocaine doses (ps ≤ 0.001). Rats treated with d-amphetamine during adolescence earned fewer cocaine infusions than vehicle-

treated rats at 0.3 and 1.0 mg/kg cocaine ($p \leq 0.05$ and 0.01, respectively). More specifically, adolescent d-amphetamine reduced cocaine intake in adult WIS at 0.3 and 1.0 mg/kg cocaine ($p \leq 0.003$ and 0.001, respectively), and did not significantly alter cocaine intake in adult WKY or SHR.

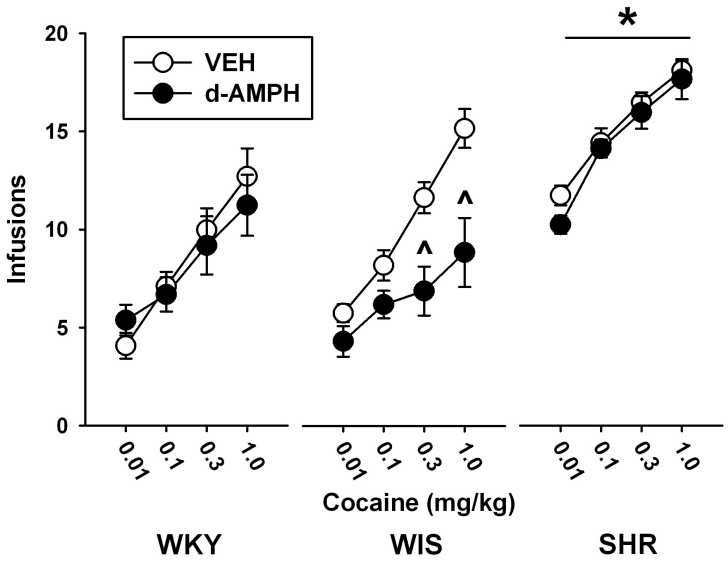

Figure 30. Cocaine dose-response functions, based on infusions earned under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

The PR cocaine dose-response functions based on active lever responses are shown in Figure 31. For the three-way ANOVA, differences in strain ($F(2, 37) = 39$, $p \leq 0.001$) and dose ($F(3, 111) = 62.7$, $p \leq 0.001$) were observed, as well as a strain X dose interaction ($F(6, 111) = 6.3$, $p \leq 0.001$). SHR made more active lever responses than WKY and WIS (all dose ps ≤ 0.001, except for 0.01 mg/kg where ps ≤ 0.06). Rats treated

with d-amphetamine during adolescence made fewer active lever responses than vehicle-treated rats at 1.0 mg/kg cocaine ($p \leq 0.02$). More specifically, adolescent d-amphetamine reduced active lever responding in adult WIS at 1.0 mg/kg cocaine ($p \leq 0.001$). There was also a trend towards reduced active lever responding at 0.3 mg/kg cocaine in d-amphetamine-treated WIS ($p \leq 0.07$). Adolescent d-amphetamine did not alter active lever responding in adult WKY or SHR.

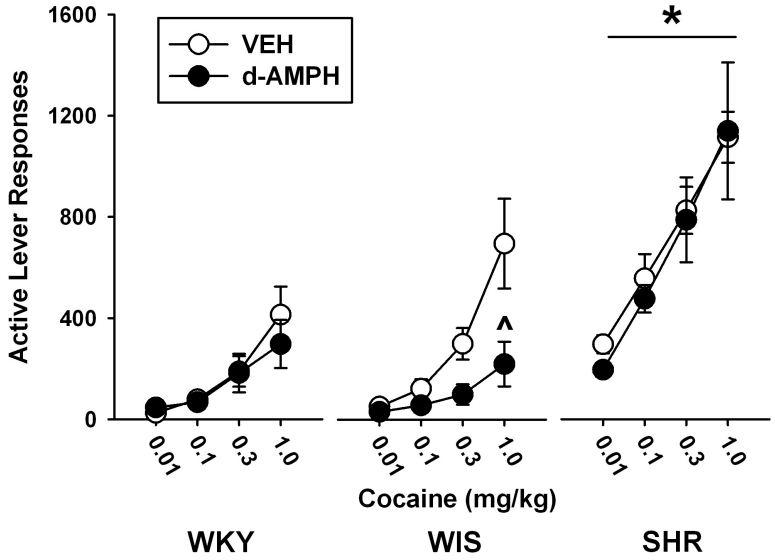

Figure 31. Cocaine dose-response functions, based on active lever responding under a PR schedule of reinforcement, by adult WKY, WIS, and SHR following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

For inactive lever responding under the PR schedule, there was a main effect of dose ($F(3, 111) = 2.6$, $p \leq 0.05$), although post-hoc testing did not reveal any significant differences between cocaine doses. There was also a trend towards a strain difference in

inactive lever responding ($F(2, 37) = 2.8, p \leq 0.07$). Further testing revealed that SHR overall made more inactive responses (111.8 ± 48) than WKY ($7.8 \pm 1.4, p \leq 0.05$).

Experiment 9: Cocaine Cue Reactivity and Relapse Behavior in Adulthood Following Adolescent d-Amphetamine Treatment

Experiment 9 sought to reproduce findings in Experiment 4, showing that adult SHR are more reactive to cocaine-related cues than WKY and WIS, such that SHR emit more cocaine-seeking responses under a second-order schedule of cocaine delivery and cue presentation. Experiment 9 also tested the hypothesis that treatment with a clinically relevant dose of d-amphetamine during adolescence does not increase cocaine cue reactivity in adult SHR, after adolescent treatment is discontinued.

Materials and Methods

Experiment 9a: Maintenance Testing under a Second-Order Schedule.

Experiment 9 involved animals from Experiment 5, which underwent strategy set shift testing and were treated with d-amphetamine or saline during adolescence. Surgical and cocaine self-administration procedures were identical to those followed in Experiment 4, except that rats in Experiment 9 did not require lever training for food to facilitate acquisition of responding, due to prior lever-pressing experience in Experiment 5. Briefly, on P67, animals completing Experiment 5 underwent surgical implantation of i.v. catheters. On P77, following surgical recovery, rats began self-administration training for

0.3 mg/kg cocaine under an FR1 schedule, and were incrementally trained to a terminal fixed-interval (FI)-based second-order schedule, designated FI 5-min [FR5:S]. Training on the second-order schedule continued until rats reached stable levels of responding, designated the maintenance testing phase. Final group sizes for Experiment 9 were $n = 8$ for vehicle- and d-amphetamine-treated WKY and WIS as well as for vehicle-treated SHR; the group size for d-amphetamine-treated SHR was $n = 9$.

Experiment 9b: Extinction Training. Following completion of the maintenance testing phase, rats underwent response extinction training as described in Experiment 4b. Extinction training continued for a minimum of 10 sessions or until criterion was reached, defined as active lever responding that was ≤ 10% of the maintenance baseline for 3 consecutive sessions (constituting the extinction baseline). If rats did not reach criterion, a maximum of 21 extinction sessions was imposed.

Experiment 9c: Cue-Induced Reinstatement Testing. Following achievement of extinction criterion, rats underwent cue-induced reinstatement testing as described in Experiment 4c. All rats underwent 7 reinstatement sessions. At the end of Experiment 9c, all animals were humanely euthanized with an overdose of sodium pentobarbital (Euthasol).

Data Analysis. Dependent measures in Experiment 9 included the number of cocaine infusions earned, active and inactive lever responses, and the number of sessions

required to reach extinction criterion. Measures were analyzed by three-factor (strain X treatment X reinstatement session, or strain X treatment X phase) ANOVAs, with repeated measures for session or phase (extinction vs. reinstatement), followed by post-hoc Tukey tests.

Results

Experiment 9a: Maintenance Testing under a Second-Order Schedule. Cocaine intake during maintenance testing under the second-order schedule is depicted in Figure 32. Strains differed in cocaine intake ($F(2, 43) = 12.3$, $p \leq 0.001$), with adult SHR earning more cocaine infusions than WKY and WIS control strains ($p \leq 0.001$ and 0.03, respectively). Main and interaction effects of treatment were not significant, and further analysis confirmed that adolescent d-amphetamine treatment did not alter cocaine intake in any strain during maintenance testing.

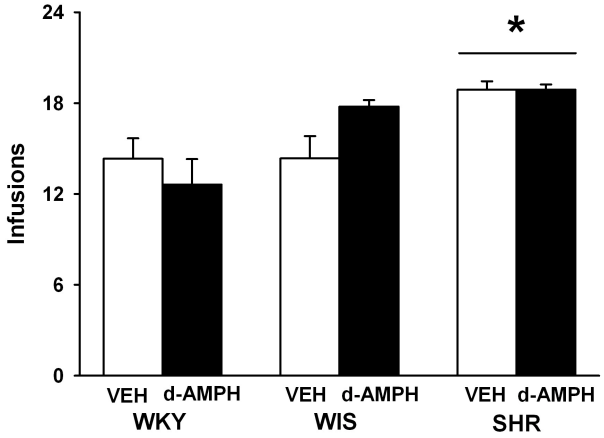

Figure 32. Cocaine intake during maintenance testing under a second-order schedule of reinforcement in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Active lever responses during maintenance testing and during the first drug-free interval of the final maintenance testing session are shown in Figures 33 and 34, respectively. Analysis of active lever responding during maintenance testing revealed strain differences ($F(2, 43) = 13.6$, $p \leq 0.001$). Overall, adult SHR made more active lever responses than WKY and WIS control strains ($p \leq 0.001$ and 0.005, respectively). During the first drug-free interval, there was also a main effect of strain ($F(2, 43) = 20.2$, $p \leq 0.001$). Adult SHR again made more active lever responses than WKY and WIS control ($p \leq 0.005$ and 0.001, respectively). Main and interaction effects of treatment were not significant. Further analysis additionally confirmed that adolescent d-amphetamine treatment did not alter cocaine seeking in any strain during maintenance testing. Inactive lever responses also did not differ by strain or treatment.

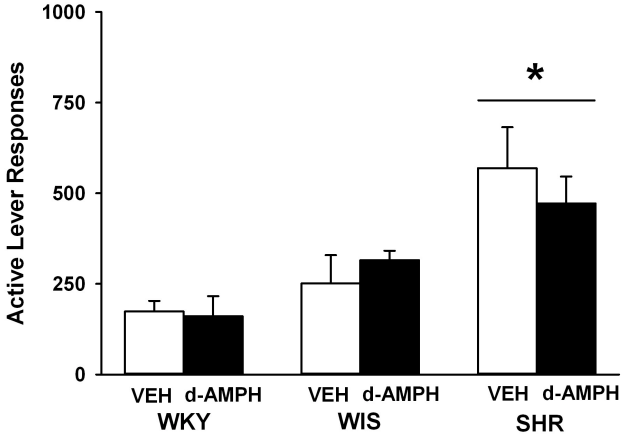

Figure 33. Active lever responding averaged across the maintenance testing baseline in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

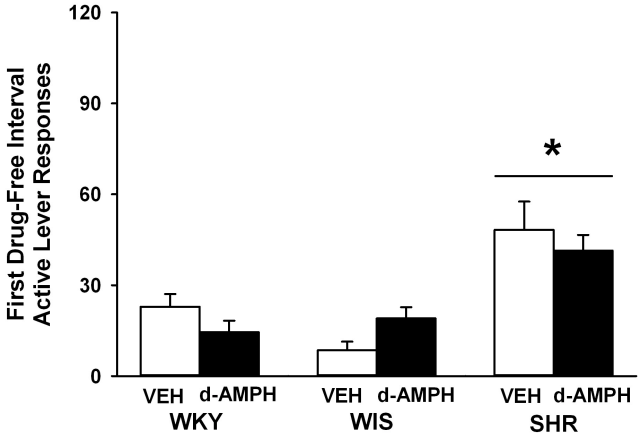

Figure 34. Active lever responding during the first drug-free interval of the final maintenance testing session in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Experiment 9b: Extinction Training. The number of sessions required for rats to reach extinction criterion is shown in Figure 35. Strains differed in the number of sessions ($F(2, 43) = 8.1, p \leq 0.001$), with SHR requiring more sessions to extinguish responding than WKY or WIS controls ($p \leq 0.01$ and 0.001, respectively). Analysis of the extinction baseline (Figure 36) also revealed strain differences ($F(2, 43) = 4, p \leq 0.02$). SHR and inbred WKY controls made relatively more active lever responses than the outbred WIS control strain during the extinction baseline ($p \leq 0.05$ and 0.04, respectively). However, all strains extinguished to less than 10% of the maintenance baseline level of responding. Inactive lever responses during the extinction baseline also differed by strain ($F(2, 43) = 3.7, p \leq 0.03$), with SHR making more inactive responses (31 ± 12) than WIS (6 ± 1; $p \leq 0.03$), but not WKY (9 ± 2). Adolescent d-amphetamine treatment did not alter any measure across strains during extinction training in adult rats.

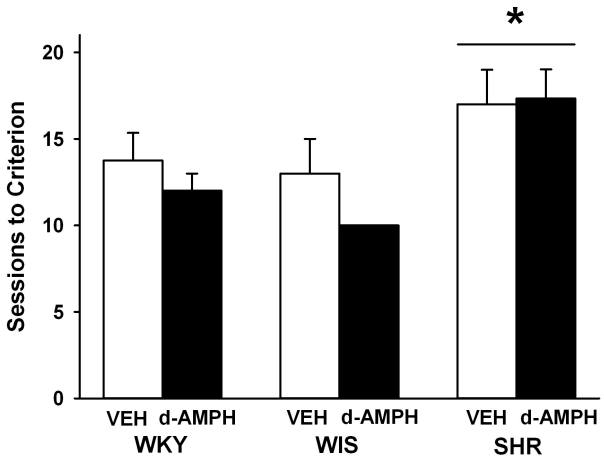

Figure 35. Number of sessions required to reach extinction criterion in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

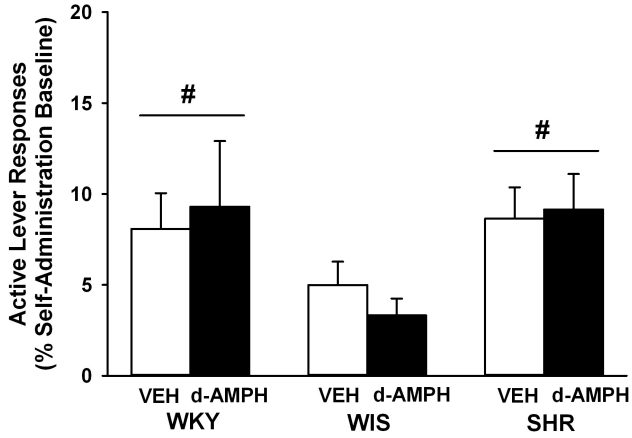

Figure 36. Active lever responding during the extinction baseline, expressed as a percentage of the maintenance testing baseline, in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence (Mean ± SEM). # $p \leq 0.05$ compared to WIS overall.

Experiment 9c: Cue-Induced Reinstatement Testing. The number of active lever responses during reinstatement testing, averaged across the seven sessions of testing as well as during the first hour of the extinction baseline for comparison, is shown in Figure 37. Three-way ANOVA revealed a main effect of phase ($F(1, 43) = 116$, $p \leq 0.001$) and strain ($F(2, 43) = 41.2$, $p \leq 0.001$), as well as a strain X phase interaction ($F(2, 43) = 19.5$, $p \leq 0.001$). Post-hoc testing of the interaction indicated that cue re-exposure reinstated cocaine-seeking responses above extinction levels in all groups ($ps \leq 0.001$). Adult SHR reinstated more cocaine-seeking responses than WKY and WIS ($ps \leq 0.001$). SHR also emitted more responses during the first hour of the extinction baseline than WKY and WIS ($ps \leq 0.03$). There were no treatment differences in active lever responses averaged across the seven sessions of reinstatement testing.

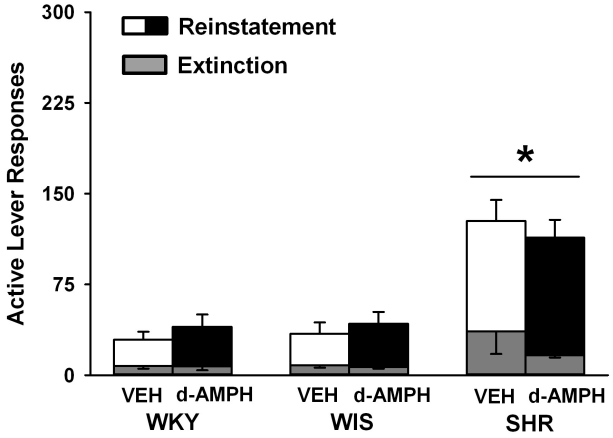

Figure 37. Active lever responding averaged across the seven reinstatement test sessions in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence. For comparison, active lever responses during the first hour of extinction training were averaged across the extinction baseline and are depicted in the gray bars (Mean ± SEM). * $p \leq 0.05$ compared to both other strains.

Effects of d-amphetamine emerged when cocaine-seeking responses were analyzed within the seven individual reinstatement test sessions, as shown in Figure 38. Three-way ANOVA revealed a main effect of strain ($F(2, 42) = 32.8$, $p \leq 0.001$) and reinstatement session ($F(6, 252) = 2.5$, $p \leq 0.02$). Further testing revealed that, during session 1 of reinstatement, SHR reinstated more active lever responses than the WKY and WIS controls overall ($p \leq 0.001$). Moreover, adult SHR treated with adolescent d-amphetamine reinstated fewer active lever responses than SHR treated with vehicle ($p \leq 0.003$). However, this reduction was not maintained across reinstatement sessions in amphetamine-treated SHR. For each subsequent session of reinstatement testing, strain differences were sustained (SHR > WKY = WIS; ps ≤ 0.008). In WKY or WIS,

adolescent d-amphetamine treatment did not alter active lever responses during session 1 or on any other reinstatement test session.

For inactive lever responses, three-way ANOVA revealed a session X strain X treatment interaction ($F(12, 252) = 2.2$, $p \leq 0.01$). Further testing indicated that, overall, SHR made more inactive lever responses than the WKY and WIS controls ($p \leq 0.03$). Moreover, during session 1 of reinstatement, adult SHR treated with adolescent d-amphetamine made fewer inactive lever responses than SHR treated with vehicle ($p \leq 0.006$). In WKY and WIS, adolescent d-amphetamine treatment did not alter inactive lever responses during session 1 or on any other reinstatement test session.

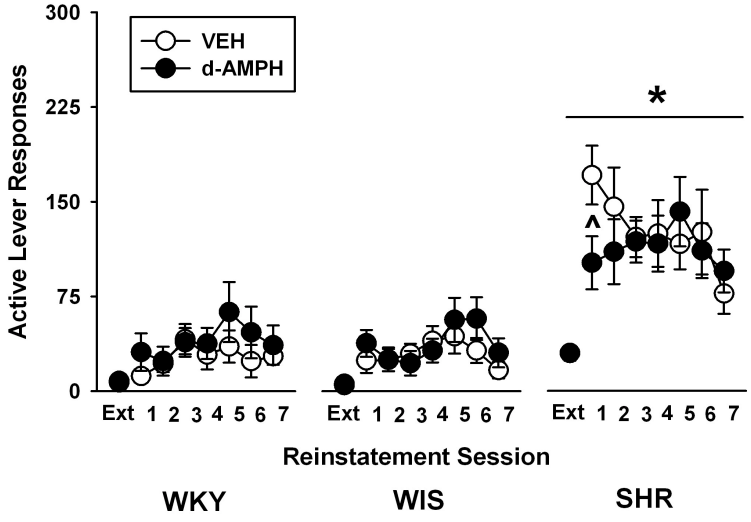

Figure 38. Active lever responses during each reinstatement test session in adult WKY, WIS, and SHR, following vehicle or d-amphetamine treatment during adolescence. For comparison, active lever responses during the first hour of extinction (Ext) training, averaged across the extinction baseline, are also shown (Mean ± SEM). * $p \leq 0.05$ compared to both other strains. ^ $p \leq 0.05$ compared to vehicle treatment in the same strain.

Discussion

Strain Differences in Prefrontal Cortex Functioning in Adolescence

Experiment 5 employed an operant version of the strategy set-shifting task to assess PFC functioning during adolescence in the SHR model of ADHD. During the initial set formation, SHR exhibited slower and more variable reaction times than WIS. These results are consistent with observations in individuals with ADHD who demonstrate high between-subject reaction time variability, and in some studies, slower reaction time, compared to controls without ADHD (Kofler et al, 2013). High reaction time variability is, moreover, a robust and highly replicable feature of ADHD and may contribute to other symptoms, such as poor sustained attention, behavioral inhibition, and working memory deficits (Kofler et al, 2013). Interestingly, these symptoms have been attributed to altered frontal cortex and DAT functioning (Kofler et al, 2013; MacDonald et al, 2006), which also are observed in SHR (Li et al, 2007; Mill et al, 2005; Miller et al, 2014; Roessner et al, 2010; Watanabe et al, 1997).

During the initial set formation, adolescent SHR did not show deficits in speed of learning or choice accuracy, or have a greater number of trial omissions relative to WKY or WIS, consistent with prior work (Harvey et al, 2013). It is possible that SHR more readily respond than WKY or WIS for reinforcement under task conditions that do not require complex behavioral flexibility, such as during acquisition of the initial attentional set (Harvey et al, 2013; Johansen et al, 2002). However, WKY made more trial omissions and had slower and more variable reaction times than WIS, and took longer to reach

learning criterion than both WIS and SHR. The latter finding was observed both in the current investigation and in prior work (Harvey et al, 2013). Therefore, it is possible that adolescent WKY are less proficient in their ability to acquire the initial set relative to the other two strains. Although WKY are frequently used as an inbred control strain for SHR (Russell et al, 2005), the WKY, particularly from Charles River Laboratories in the USA, also have been used as a model of anxiety, depression, and more recently, autism spectrum disorder (Paré et al, 1994; Paré & Kluczynski, 1997; Zhang-James et al, 2014). Compared to outbred rat strains, WKY show reduced locomotor activity and social exploratory behavior in the open field test, elevated plus maze, and three-chamber social interaction test, as well as increased learned helplessness and immobility in forced swim and defensive burying paradigms (Pardon et al, 2002; Paré et al, 1994; Zhang-James et al, 2014). Furthermore, in reinforcement-based tasks, WKY earn fewer sucrose pellets than WIS under FR1 and PR schedules (De La Garza II, 2005). Behavioral abnormalities exhibited by WKY have been attributed to reduced cortical and hippocampal volume and plasticity, decreased D1 receptor and DAT binding in striatum, and decreased dopamine and dopamine metabolite levels in PFC relative to outbred rat strains (Cominski et al, 2014; De La Garza II & Mahoney III, 2004; Jiao et al, 2003; Novick et al, 2008). Importantly, the initial set formation of the strategy set shifting task is dependent on dopaminergic projections to frontal cortex as a whole, as 6-OHDA lesions in frontal cortex, but not caudate nucleus, impaired acquisition of an initial set (Crofts et al, 2001). Decreased dopamine levels in frontal cortex of WKY (De La Garza II & Mahoney III, 2004) may therefore contribute to slower learning of the initial strategy set in this strain.

Although the WKY have been proposed to be the most appropriate comparator strain for SHR (Harvey et al, 2013; Russell et al, 2005), these findings emphasize the importance of concurrent evaluation of WKY and an outbred control when examining set shifting performance in SHR.

In contrast to the initial set formation, the set shift phase of the task necessitates behavioral flexibility, requiring rats to switch responding from a previously learned visual strategy to a spatial strategy. Consistent with previous studies (Harvey et al, 2013) and as hypothesized, adolescent SHR in the current study showed the greatest deficits during the set shift phase. Specifically, SHR exhibited slower and more variable reaction times, and made more trial omissions and regressive errors than both control strains. On the Wisconsin Card Sorting Task, individuals with ADHD exhibit poor behavioral flexibility by making more perseverative responses and completing fewer categories than typically developing controls (Kado et al, 2012; Reeve & Schandler, 2001; Seidman et al, 1997). Poor behavioral flexibility and other ADHD-related symptoms, such as hyperactivity, inattention, low motivation, and poor motor timing and response inhibition, may arise from abnormal neural activation and connectivity in frontostriatal circuitry both in humans and in SHR (Cubillo et al, 2012; Rubia et al, 2011; Warton et al, 2009). For example, as reviewed in Chapter One, individuals with ADHD exhibit elevated frontostriatal glutamate transmission (Courvoisie et al, 2004; MacMaster et al, 2003), and SHR exhibit greater potassium-evoked glutamate release in PFC (Miller et al, 2014). Moreover, glutamate application to substantia nigra, which receives excitatory projections from mPFC, results in greater dopamine release in the substantia nigra of

SHR compared to controls (Warton et al, 2009). Elevated dopamine activity in substantia nigra and striatum may also contribute to other neurochemical and genetic abnormalities shared by SHR and individuals with ADHD. These include increased DAT expression in striatum (Faraone et al, 2005; Mill et al, 2005; Roessner et al, 2010; Watanabe et al, 1997) and decreased dopamine D4 receptor gene expression and protein synthesis in PFC, the latter of which is involved in regulating PFC glutamate activity (Li et al, 2007; Miller et al, 2014). Because many studies investigating the neurobiological correlates of the ADHD phenotype in SHR have employed WKY as the sole comparator strain, these comparisons should be interpreted cautiously. Nonetheless, altered frontostriatal glutamate and dopamine activity, among other neurochemical abnormalities, likely contribute to poor SHR performance on the set shift. Previous studies have established that dopamine and glutamate interactions in mPFC are critical for behavioral flexibility and strategy switching (Floresco et al, 2008) and that dopamine and glutamate interactions in substantia nigra are critical for initiation of locomotor activity (Warton et al, 2009).

During the set shift phase, WKY reached learning criterion faster than both WIS and SHR and made fewer errors than WIS. It is possible that WKY exhibit superior behavioral flexibility in strategy shifting compared to WIS or SHR. However, given that WKY were slower in acquiring the initial set, it is also possible that WKY experienced less proactive interference during the set shift than WIS or SHR. Proactive interference from a previously relevant stimulus dimension exacerbates cognitive inflexibility when individuals are required to attend to a new stimulus feature (Dick, 2012). Previous work

identified reduced proactive interference in WKY in an eyeblink conditioning paradigm, whereby pre-exposure to unconditioned or conditioned stimuli did not alter acquisition rate of the eyeblink response in WKY, but slowed acquisition in outbred Sprague-Dawley rats (Ricart et al, 2015). Though it is unclear to what degree proactive interference in classical conditioning generalizes to operant conditioning, it is plausible that weakened acquisition, memory storage, or retrieval of the initially reinforced visual strategy by WKY in the current study could lead to reduced proactive interference during subsequent testing phases. This would be manifested as fewer errors and an apparently faster ability to acquire new response strategies (Kantak et al, 2014).

Reduced proactive interference may also help to explain some of the current observations during the reversal learning phase of the set shift task. During reversal learning, SHR continued to show slower and more variable reaction times, consistent with the ADHD phenotype. However, WKY and SHR reached the reversal learning criterion faster than WIS. Moreover, during this phase WKY made fewer trial omissions and SHR made fewer perseverative errors than WIS rats. It is possible that, relative to SHR and WKY, WIS exhibit deficits in reversal learning, which is dependent on OFC functioning (Ghods-Sharifi et al, 2008). However, given the behavioral and neurobiological abnormalities in WKY and SHR reviewed above, an alternative explanation is that WKY and SHR experienced less proactive interference than WIS during reversal learning. Deficient learning or memory of the previously acquired behavioral strategies by WKY and SHR would lead to the appearance of superior performance relative to WIS during the reversal learning phase. Conversely, normative

learning and retrieval of the previously acquired behavioral strategy by WIS would lead to increased proactive interference, resulting in slower speed of reversal learning and a greater number of perseverative errors as observed in the current study.

Effects of d-Amphetamine Treatment on Prefrontal Cortex Functioning in Adolescence

As hypothesized, d-amphetamine improved several behavioral measures in adolescent SHR during the set shift phase. Specifically, SHR treated with d-amphetamine reached the set shift learning criterion faster, were more accurate in response choices, and made fewer regressive and never-reinforced errors than vehicle-treated SHR. Although d-amphetamine did not improve reaction time or reaction time variability in SHR during any phase of the set shift task, it is rare that all ADHD symptoms are alleviated by single drug therapy (Wilens et al, 2009). It is possible that a higher d-amphetamine dose would improve additional ADHD-related deficits in SHR. For example, d-amphetamine doses of 0.64, 1.27, and 1.91 mg/kg improved hyperactivity and sustained attention in adult SHR, but only 1.27 and 1.91 mg/kg of d-amphetamine improved impulsivity in adult SHR (Sagvolden & Xu, 2008). However, these higher doses are also likely to produce plasma levels and monoamine responses that exceed clinical relevance, in contrast to the 0.5 mg/kg dose used in the current work (Heijtz et al, 2003). Nonetheless, the current findings indicate that d-amphetamine selectively improves behavioral flexibility in SHR without altering performance in WKY or WIS control strains.

Strain Differences in Cocaine Abuse Risk and Cocaine Cue Reactivity in Adulthood

In Experiment 6, adult SHR spontaneously acquired cocaine self-administration faster, earned more cocaine infusions, and made more cocaine-maintained responses than adult WKY and WIS controls, consistent with the findings in Aim 1. SHR also exhibited increased cocaine abuse risk in Experiments 7 and 8, as reflected by vertical (upward) shifts in FR1 and PR cocaine dose-response functions. These results indicate that the efficacy and motivating influence of cocaine reinforcement is augmented in SHR, in accord with the results of Aim 1 and with prior studies (Harvey et al, 2011; Piazza et al, 2000; Somkuwar et al, 2013a). Moreover, Experiment 9 reproduced findings showing that SHR were more reactive to cocaine-related cues than WKY and WIS, both when cocaine was available (maintenance testing) and was not available (reinstatement testing) for self-administration.

One notable difference across experiments is that SHR took longer to extinguish lever responding than WKY and WIS in Experiment 9. Conversely, in Experiment 4, both SHR and WKY took longer to extinguish lever responding than WIS. The latter result was interpreted to suggest that extinction learning was augmented in the WIS strain, rather than SHR exhibiting an extinction deficit. However, other studies have suggested that, like individuals with ADHD, SHR exhibit slower extinction rates than controls (Brackney et al, 2012; Kollins et al, 1997). Moreover, slower extinction in SHR may be a result of abnormal nigrostriatal dopamine functioning that conveys low sensitivity to changes in reinforcement contingencies, a deficit also found in individuals

with ADHD (Kollins et al, 1997; Sagvolden et al, 2005). The findings of Experiment 9, in conjunction with prior work, suggest that SHR do exhibit deficits in extinction learning. Nonetheless, SHR did not differ from WKY in the relative magnitude to which responding was extinguished, and all strains and treatment groups extinguished active lever responding to ≤ 10% of the self-administration baselines, on average. High levels of cocaine seeking by SHR during reinstatement testing thus remain reflective of heightened cocaine cue reactivity in this preclinical model of ADHD.

Another notable behavioral difference in Experiment 9 vs. Experiment 4 was that both vehicle- and drug-treated rats of WKY, WIS, and SHR strains exhibited lower rates of lever responding overall in Experiment 9. This may relate to the fact that the adult rats used in Experiment 9 had prior lever training experience during adolescence, and exposure to chocolate pellet reinforcers, from engaging in the strategy set shifting task (Experiment 5). In contrast, the adult rats used in Experiment 4 were naïve to operant chambers and had no lever training experience until adulthood. Interestingly, a similar effect was observed in experiments evaluating methylphenidate and atomoxetine treatments, wherein adult rats that participated in strategy set shifting during adolescence exhibited reduced rates of lever responding in adulthood, compared to rats that were naïve to operant chambers until adulthood (Jordan et al, 2014). Together, these findings suggest that lever pressing or reinforcer experience during adolescence may influence the overall magnitude of responding for cocaine under the second-order schedule. However, despite these differences in overall rates of responding, the same relative magnitude of strain differences was observed in all experiments. That is, SHR consistently made more

cocaine-seeking responses and earned more cocaine infusions than WKY and WIS control strains. The fact that these strain differences have been reproduced in multiple experiments run at different time points and, in the case of the methylphenidate, by a different investigator, indicates that the findings are highly robust, and further emphasizes that the SHR is a valuable model of comorbid ADHD, cocaine abuse, and heightened cocaine cue reactivity.

In addition to elevated cocaine abuse risk and cue reactivity, SHR generally exhibited increased inactive lever responding compared to control strains, particularly relative to the inbred WKY. Notably, hyperactivity in the SHR has previously been criticized as apparent only relative to WKY, which exhibits reduced locomotor activity compared to outbred rats in a number of behavioral tests (Kantak et al, 2008; Pardon et al, 2002; Paré & Kluczynski, 1997; Zhang-James et al, 2014). However, in the current work, WKY and WIS did not differ on any measure of cocaine self-administration, with the exception of modest differences during extinction training and responding for cocaine under the FR1 schedule. These observations contradict the possibility that WKY hypoactivity influenced the current findings. Nonetheless, these findings again emphasize the importance of concurrent evaluation of WKY and an outbred control when examining set shifting performance in SHR.

Effects of Adolescent d-Amphetamine Treatment on Cocaine Abuse Risk and Cue Reactivity in Adulthood

As hypothesized, adolescent d-amphetamine treatment did not increase cocaine abuse risk in adult rats, but was instead protective in SHR and WIS across several measures. Specifically, in adult SHR, adolescent d-amphetamine reduced cocaine intake and active lever responding during acquisition of cocaine self-administration, and modestly reduced cocaine cue reactivity on the first day of reinstatement testing. The effects of adolescent atomoxetine treatment were similar, except that atomoxetine reduced cocaine-seeking responses across the entire seven-day reinstatement test phase in adult SHR. In contrast, adolescent methylphenidate treatment augmented multiple aspects of cocaine abuse risk in SHR, as described in the preceding chapters (Baskin et al, 2015; Harvey et al, 2011; Jordan et al, 2014). These findings suggest that d-amphetamine is a viable alternative to methylphenidate for teenagers with ADHD who are initiating treatment.

Surprisingly, d-amphetamine had the most robust effects on cocaine abuse risk in adult WIS. In this outbred control strain, adolescent d-amphetamine treatment delayed acquisition of cocaine self-administration, and reduced cocaine intake as well as active lever responding under FR1 and PR schedules of reinforcement in adulthood. Downward shifts in cocaine dose-response functions reflect reductions in the efficacy and motivating influence of cocaine reinforcement (Piazza et al, 2000). Notably, these effects were observed months after d-amphetamine treatment had been discontinued. Although

adolescent methylphenidate did not alter FR1 or PR dose-response functions in adult WIS, it decreased the speed to acquire cocaine self-administration in this strain, similar to d-amphetamine (Harvey et al, 2011).

In contrast to the moderately beneficial effects of adolescent d-amphetamine in SHR and WIS, d-amphetamine treatment speeded acquisition of cocaine self-administration in adult WKY. Interestingly, adolescent atomoxetine treatment also speeded acquisition of cocaine self-administration in adult WKY, whereas adolescent methylphenidate had no effect on cocaine abuse risk in this strain (Harvey et al, 2011; Somkuwar et al, 2013a). Fortunately, these medications did not alter any other aspects of cocaine abuse risk in WKY. The observations of altered cocaine abuse risk following adolescent d-amphetamine treatment in WKY and WIS further emphasize the necessity of assessing both inbred and outbred control strains when examining cocaine abuse risk in the SHR, as well as the importance of accurate diagnosis of ADHD. The implication of these findings, as well as the neurochemical mechanisms that may contribute to the effects of adolescent ADHD medications in these three strains, are explored in greater detail in Chapter Four.

CHAPTER FOUR.

General Discussion

This dissertation sought to identify alternative medications to methylphenidate that would not increase cocaine abuse risk in newly diagnosed teenagers with ADHD, using a preclinical model. Experiments in Aim 1 focused on the effects of the non-stimulant medication, atomoxetine, which inhibits NET function. Experiments in Aim 2 focused on an alternative stimulant medication, d-amphetamine, which a different mechanism of action than methylphenidate. The significance of the findings is discussed in the subsequent sections.

Importance of the Spontaneously Hypertensive Rat Model of ADHD

The results of this dissertation confirm and extend prior studies on cognitive functioning and cocaine abuse risk in the SHR model. For example, adolescent SHR exhibited deficits in behavioral flexibility during the set shift phase of the strategy set shifting task, consistent with prior studies (Harvey et al, 2013). Adolescent SHR also exhibited deficits in reaction time and reaction time variability during the initial set formation and reversal learning, compared to control strains. High reaction time variability is a novel finding in adolescent SHR, and is a well-reproduced feature of ADHD (Kofler et al, 2013). This finding thus further verifies the utility of adolescent SHR as a preclinical model of ADHD.

During adulthood, SHR earned more cocaine infusions and made more active lever responses for cocaine than control strains throughout all experiments. More specifically, SHR acquired cocaine self-administration faster and were more sensitive to the efficacy and motivating influence of cocaine reinforcement, reflected in upward shifts in FR1 and PR cocaine dose-response functions, consistent with prior studies (Harvey et al, 2011). The novel finding of the current work regarding cocaine abuse risk is that SHR were more reactive to cocaine-related cues. High cue reactivity in SHR was found when cocaine was available (maintenance testing) and was not available (reinstatement testing and the first drug-free interval of maintenance testing) for self-administration. Moreover, the current work also demonstrated that SHR take longer to extinguish lever responding, consistent with observations in ADHD (Brackney et al, 2012; Kollins et al, 1997).

Sign tracking, a Pavlovian conditioned approach behavior associated with drug abuse (Tomie et al, 2008), may contribute to heightened incentive salience of cocaine-associated cues in animals that are vulnerable to cocaine addiction, such as the SHR (Yager & Robinson, 2013). Whereas goal tracking involves approach to an unconditioned stimulus, sign tracking involves compulsive approach to reward-related cues. Although Pavlovian conditioned approach behavior has not been evaluated directly in SHR, ADHD-related characteristics, such as impulsivity and poor sustained attention, are observed in SHR as well as in sign tracking rats (Sagvolden et al, 2005; Tomie et al, 2008; Wooters & Bardo, 2011). Thus, it is possible that SHR also have a sign tracking phenotype, given their high degree of cocaine cue reactivity. Although further studies on cocaine cue reactivity in ADHD are needed, if the current findings in SHR are

translational, substance-dependent individuals with ADHD may uniquely benefit from cue-exposure therapy (Mitchell et al, 2013), which seeks to extinguish the saliency of drug-related cues, and is most effective in individuals with initially high cue reactivity (Unrod et al, 2013). Moreover, pharmacotherapies that enhance extinction of drug-related cues by targeting glutamate signaling (Achat-Mendes et al, 2012; Kaplan et al, 2011) may have relevance for individuals with ADHD, who exhibit altered frontostriatal glutamate activity (Courvoisie et al, 2004; MacMaster et al, 2003).

In summary, this dissertation further confirms that value of the SHR as a model of comorbid ADHD and cocaine abuse risk. Given the increase in cocaine abuse risk conveyed by adolescent methylphenidate treatment in SHR (Baskin et al, 2015; Harvey et al, 2011; Jordan et al, 2014), it is important to identify other medications for teenagers with ADHD.

Significance of Adolescent Atomoxetine Treatment on Cocaine Abuse Risk in Adulthood

Experiments 1 through 4 examined the effects of administration of a therapeutically relevant dose of atomoxetine during adolescence on cocaine abuse risk in adulthood. The results of these experiments demonstrate that, in contrast to methylphenidate, adolescent atomoxetine treatment does not increase cocaine abuse risk in adult SHR, and may even modestly reduce cocaine cue reactivity in this strain. The effects of adolescent atomoxetine treatment on monoamine transporter function and expression in PFC may help to explain these findings, as summarized in Tables 1 through 3. For example, OFC activation plays a critical role in cocaine seeking and cue reactivity

(Di Pietro et al, 2008; Kantak et al, 2009; Nelissen et al, 2012). One past study demonstrated that daily atomoxetine (1 mg/kg) during late adolescence (P40 - 54) increased NET mRNA in the OFC of adult outbred rats (Sun et al, 2012). However, mRNA levels often do not correlate with protein expression (Greenbaum et al, 2003). A more recent study demonstrated that daily atomoxetine (3 mg/kg) during adolescence (P21 - 42) decreased NET protein levels in the hippocampus of outbred rats, for up to 2 months after treatment was discontinued (Udvardi et al, 2013). Both studies inferred an inhibitory effect of atomoxetine on neural signaling: the 1 mg/kg treatment regimen decreased synaptic plasticity markers in the OFC, including BDNF mRNA and phosphorylated CREB and ERK (Sun et al, 2012), and the 3 mg/kg treatment regimen decreased glutamate NMDA receptor subunit levels in the hippocampus and striatum (Udvardi et al, 2013). While it remains to be determined if adolescent treatment with 0.3 mg/kg atomoxetine decreases NET function or has an inhibitory effect on OFC in adult SHR, these mechanisms may contribute to the modest decrease in cocaine cue reactivity observed in adult SHR when cocaine was not available for self-administration. In addition, chronic treatment with 0.3 mg/kg of atomoxetine during adolescence decreases DAT function and expression in the OFC of adult SHR (Somkuwar et al, 2013a), which may also contribute to the reduction in cocaine cue reactivity. Atomoxetine treatment during adolescence similarly decreased DAT function in OFC of adult WIS (Somkuwar et al, 2013a), and in the current work did not alter any aspect of cocaine abuse risk in this strain.

Another variable that may confer protection against an increase in cocaine abuse risk is the failure of adolescent atomoxetine to augment DAT function in the mPFC in adulthood (Somkuwar et al, 2013a). In previous studies, further increases in the speed to acquire cocaine self-administration, the efficacy and motivating influence of cocaine reinforcement, and cocaine intake was observed following discontinuation of adolescent methylphenidate vs. vehicle treatment in adult SHR (Harvey et al, 2011; Jordan et al, 2014). The same adolescent methylphenidate treatment regimen also selectively increased DAT function in mPFC of adult SHR (Somkuwar et al, 2013b). As reviewed in Chapter One, an increase in mPFC DAT function may contribute to an enhanced reinforcing value of cocaine. Notably, in contrast to its effects on cocaine intake, adolescent methylphenidate treatment did not alter cocaine seeking during maintenance or reinstatement testing and did not alter the number of sessions required to reach the extinction criterion in adult SHR (Jordan et al, 2014). Because adolescent methylphenidate further increased cocaine intake in SHR, it might be expected that cue reactivity also would be further increased. However, these results were consistent with prior findings in outbred rats showing that acute administration of methylphenidate did not alter cocaine seeking under a second-order schedule (Economidou et al, 2011).

In summary, although additional work is needed to confirm the mechanisms and signaling pathways involved, the protective effect of atomoxetine against increases in cocaine abuse risk likely relates to long-lasting effects on NET and DAT function in OFC, and a lack of effect on DAT function in mPFC. These findings suggest that

adolescent atomoxetine treatment may represent a safer medication than methylphenidate for newly diagnosed teenagers with ADHD.

It is important to note that adolescent atomoxetine speeded acquisition of cocaine self-administration in the inbred WKY control strain. Consistent with the proposed role of DAT in cocaine abuse risk, adolescent atomoxetine treatment increases DAT function in OFC of WKY (Somkuwar et al, 2013a). This neurochemical adaptation may contribute to an enhanced reinforcing value of cocaine in WKY when cocaine first became available for self-administration, by increasing the post-synaptic response to phasically released dopamine under conditions of low dopaminergic tone (Grace, 2001). WKY exhibit several abnormalities in monoamine transporter activity that may be uniquely altered by adolescent medications, leading to differential effects on cocaine abuse risk. For example, compared to outbred rats, WKY show decreased DAT density in the nucleus accumbens (Jiao et al, 2003), but increased NET binding in the hippocampus and amygdala (Tenjani-Butt et al, 1994). Moreover, compared to WIS, WKY exhibit reduced serotonin levels in several brain regions involved in cocaine abuse, including the amygdala, hypothalamus, nucleus accumbens, substantia nigra, and dorsal raphe nucleus (Scholl et al, 2010). Scholl and colleagues (2010) suggest that altered serotonin levels may reflect a reduction in serotonin transporters (SERT) sites in WKY. Importantly, SERT knockout speeds acquisition of psychostimulant self-administration and increases sensitivity to cocaine (Homberg et al, 2008; Oakly et al, 2014). Although atomoxetine is considered a selective NET inhibitor, evidence exists that this drug also binds to SERT (Ding et al, 2014).

Atomoxetine may therefore contribute to faster acquisition of cocaine self-administration in WKY, by exacerbating pre-existing SERT dysfunction in this strain.

While the results of Aim 1 do not critically call the into question the safety of adolescent atomoxetine, the impact of atomoxetine on cocaine abuse risk in the WKY control strain nonetheless has at least two important implications. One is that it is critical to evaluate both inbred and outbred control strains when examining the effects of adolescent medication in the SHR model of ADHD. In the current work, if WIS were the sole comparator strain, adolescent atomoxetine would appear to have few negative effects on cocaine abuse risk. Conversely, if WKY were the sole comparator strain to the SHR, adolescent atomoxetine may appear to be unsafe for teenagers without ADHD. In either case, with the use of only one control strain the consequences of adolescent atomoxetine on cocaine abuse risk would have been misrepresented.

The second implication of the results of Aim 1 is the critical importance of accurate diagnosis and treatment of ADHD. Because atomoxetine speeded acquisition of cocaine self-administration in WKY, misdiagnosis and subsequent treatment of ADHD in teenagers could result in a more rapid development of cocaine abuse. Unfortunately, there are currently no reliable biomarkers of ADHD that can be utilized in a clinical diagnosis. The development of strict diagnostic criteria and appropriate treatments for ADHD may have been hindered by skepticism surrounding ADHD as a neurological disorder in the international community (Heal et al, 2013). For example, ADHD has been criticized as an "American diagnosis," and even today remains under-diagnosed and under-treated in the United Kingdom and other countries (Heal et al, 2013). This criticism is negated by the

many genetic and neurochemical abnormalities that are associated with ADHD, which have been reviewed extensively above. Future research must therefore work towards identifying more reliable biomarkers that can be used alongside behavioral and cognitive measures to refine clinical diagnoses.

Significance of Adolescent d-Amphetamine Treatment on Cocaine Abuse Risk in Adulthood

While the results of Aim 1 suggest that atomoxetine may be a better treatment than methylphenidate for teenagers with ADHD, atomoxetine is not always as clinically efficacious as methylphenidate (Cubillo et al, 2014; Faraone et al, 2006; Sibley et al, 2014). Moreover, atomoxetine modestly worsened adolescent SHR performance during the initial phase of the strategy set-shifting task, and had non-specific effects in control strains (see Table 1; Harvey et al, 2013). Adolescent atomoxetine also increased active lever responding during acquisition of cocaine self-administration and slowed extinction learning in adult SHR. These effects are not as alarming as those observed after adolescent methylphenidate treatment. Nonetheless, it would be useful to identify an alternative medication for teenagers with ADHD that is both efficacious and does not increase cocaine abuse risk. Therefore, experiments in Aim 2 investigated a different stimulant medication, d-amphetamine.

d-Amphetamine may be more efficacious in alleviating ADHD symptoms than atomoxetine and methylphenidate (Arnold, 2000; Faraone & Buitelaar, 2010; Patrick & Morowitz, 1997; Sibley et al, 2014). Although d-amphetamine inhibits DAT and NET

like methylphenidate, it is also taken up into the cell through monoamine transporters, where it blocks VMAT-2 and binds to TAAR1 (Easton et al, 2007). Because of these additional mechanisms of action, adolescent d-amphetamine was hypothesized to have different effects on prefrontal cortex function and cocaine abuse risk than methylphenidate. Consistent with this hypothesis, d-amphetamine improved behavioral flexibility in adolescent SHR in Experiment 5, an effect that was not observed with methylphenidate treatment. In children with ADHD, treatment with d-amphetamine, as well as other ADHD medications, normalized the abnormal striatal glutamate levels that may contribute to ADHD-related symptoms in both humans and SHR (Carrey et al, 2003; Courvoisie et al, 2004; MacMaster et al, 2003; Miller et al, 2014; Spencer et al, 2013; Warton et al, 2009). However, future work is needed to determine whether d-amphetamine alters glutamate signaling in the striatum or substantia nigra of the SHR model.

In contrast to d-amphetamine, adolescent treatment with methylphenidate improved the speed of learning and choice accuracy in adolescent SHR only during the initial set formation phase (Harvey et al, 2013). The non-stimulant atomoxetine improved speed of learning and choice accuracy, and reduced trial omissions in the SHR during the set shift phase (Harvey et al, 2013), similar to the current observations with d-amphetamine. The ability of d-amphetamine to improve behavioral flexibility in adolescent SHR may relate in part to its effects not only on glutamate, but also on NET and DAT function in PFC. Like atomoxetine, d-amphetamine augments noradrenergic signaling through inhibition and as well as reversal of NET, which can improve set

shifting performance (Cain et al, 2011; Easton et al, 2007; Lapiz & Morilak, 2006; McGaughy et al, 2008). Moreover, unlike methylphenidate, chronic treatment with d-amphetamine during adolescence may decrease DAT expression and function in PFC due to amphetamine's activity at VMAT-2 and TAAR1 (Miller, 2011; Xie & Miller, 2009). Interestingly, adolescent SHR, like cocaine users, exhibit lower levels of VMAT-2 expression in the striatum compared to controls (Narendran et al, 2012; Simchon et al, 2010), which may contribute to d-amphetamine's unique effects in this strain. Nonetheless, reduced DAT expression and function after adolescent d-amphetamine treatment would result in slower clearance of dopamine and elevated dopaminergic tone in mesocortical neurons (Easton et al, 2007; Zahniser & Sorkin, 2004). Importantly, activation of post-synaptic D1 and D2 receptors in mPFC facilitates behavioral flexibility in outbred rats (Floresco, 2013; Floresco et al, 2006; Ragozzinno, 2002). Adolescent d-amphetamine may therefore produce optimal activation at D1 and D2 receptors in mPFC of SHR, improving behavioral flexibility. Although the effect of d-amphetamine on mPFC DAT function is not confirmed, recently, acute amphetamine administration (0.56 mg/kg, i.v.) was found to reduce DAT function and expression in nucleus accumbens of adult outbred rats (Ferris et al, 2015). However, additional studies are needed to test this hypothesis in mPFC of the SHR model, using a chronic dosing regimen of d-amphetamine during adolescence that is clinically relevant to ADHD. In contrast, chronic treatment with methylphenidate during adolescence increases DAT function in mPFC of SHR (Somkuwar et al, 2013b). This action potentially exacerbates pre-existing elevations

of DAT in SHR (Mill et al, 2005; Watanabe et al, 1997; Zahniser & Sorkin, 2004), and may lead to reduced dopaminergic signaling in mPFC.

In addition to improving behavioral flexibility in adolescent SHR, Experiments 6 through 9 revealed that adolescent d-amphetamine treatment does not increase cocaine abuse risk in adult SHR. Rather, adolescent d-amphetamine reduced cocaine intake during acquisition of self-administration, and, like atomoxetine, modestly decreased cocaine cue reactivity in adult SHR. As suggested above, the differential effects of adolescent d-amphetamine and methylphenidate on cocaine abuse risk in adulthood likely relate to their different long-term effects on monoamine transporter function in PFC (see Tables 1 through 3). For example, whereas adolescent methylphenidate increases mPFC DAT function and increases the reinforcing value of cocaine in SHR (Grace, 2001; Somkuwar et al, 2013b; Zahniser & Sorkin, 2004), adolescent atomoxetine and d-amphetamine may decrease DAT function in adult SHR, and consequently reduce the reinforcing value of cocaine (Ferris et al, 2015; Somkuwar et al, 2013a; Zahniser & Sorkin, 2004). It is probable that additional neurochemical adaptations contribute to the long-term effects of d-amphetamine, such as changes in post-synaptic receptors or NET and SERT, to which amphetamine also potently binds and reverses transport (Easton et al, 2007; Heal et al, 2013; Heijtz et al, 2003). Moreover, in outbred rats, early adolescent treatment with a clinically relevant dose of amphetamine has been shown to increase dendritic length, dendritic branching, and calcium/calmodulin kinase II signaling in mPFC, suggesting enhanced frontostriatal dopamine-glutamate interactions (Heijtz et al, 2003). Although future investigations are needed to determine the long-term

neurochemical effects of d-amphetamine in SHR, these findings nonetheless suggest that d-amphetamine is a viable alternative to methylphenidate for teenagers with ADHD who are initiating treatment.

The effects of adolescent d-amphetamine were even more profound in WIS. In this outbred control strain, adolescent d-amphetamine treatment slowed acquisition of cocaine self-administration and significantly reduced the efficacy and motivating influence of cocaine reinforcement, without influencing cognitive performance during adolescence. Interestingly, adolescent methylphenidate slowed acquisition speed in adult WIS, and decreased the affinity of DAT for dopamine in OFC of adult WIS (Somkuwar et al, 2013b). If adolescent d-amphetamine treatment has similar effects on reducing aspects of DAT function, as proposed above, this may contribute to the drug's protective effects against cocaine abuse risk in adult WIS.

Taken together, these results suggest that d-amphetamine may be an even more valuable medication than methylphenidate or atomoxetine for teenagers with ADHD. While concern has been raised regarding the abuse liability of d-amphetamine as a prescription medication in itself, there is little actual abuse of d-amphetamine by ADHD patients (Heal et al, 2013). The pharmacokinetics of orally administered d-amphetamine make it significantly less reinforcing than cocaine or methamphetamine, and clinically relevant doses of d-amphetamine are unlikely to produce the euphoria associated with compulsive drug use (Heal et al, 2013). Moreover, the pro-drug, lisdexamphetamine (Vyvanse®), has recently been developed to further deter abuse, by requiring enzymatic

hydrolysis within red blood cells in order to be converted into the psychoactive d-amphetamine metabolite (Heal et al, 2013; Pennick, 2010).

Interestingly, adolescent d-amphetamine, like atomoxetine, speeded acquisition of cocaine self-administration in adult WKY. As reviewed above, WKY exhibit a number of behavioral and neurochemical abnormalities that may be reminiscent of a depressive/anxiety-prone phenotype (Cominski et al, 2014; De La Garza II & Mahoney III, 2004; Jiao et al, 2003; Pardon et al, 2002; Paré et al, 2002; Novick et al, 2008; Scholl et al, 2010). The effects of adolescent d-amphetamine in WKY may relate to unique alterations in DAT and SERT function in this strain (see Tables 2 and 3; Jiao et al, 2003; Tenjani-Butt et al, 1994; Scholl et al, 2010). If the neurochemical effects of d-amphetamine resemble those of atomoxetine, d-amphetamine may produce increases in DAT function in PFC of adult WKY, thereby augmenting the reinforcing value of cocaine (Grace, 2001; Somkuwar et al, 2013a). However, the mechanism by which d-amphetamine may exert this effect on DAT in WKY is unclear. Amphetamines are also known to reduce SERT function in the striatum of outbred rats (Haughey et al, 2000; Kokoshka et al, 1998). If d-amphetamine reduces SERT function in WKY, faster acquisition of cocaine self-administration following adolescent d-amphetamine treatment may reflect an exacerbation of pre-existing reductions in SERT in this strain (Homberg et al, 2008; Oakly et al, 2014; Scholl et al, 2010). However, the effects of adolescent treatment with clinically relevant doses of d-amphetamine on SERT function in adult WKY have yet to be evaluated. Nonetheless, in conjunction with the results of Aim 1, findings in WKY emphasize the necessity of evaluating both inbred and outbred control

strains alongside the SHR. Moreover, the effects of d-amphetamine in control strains further highlight the importance of accurate diagnosis and treatment of ADHD. The long-term consequences of misdiagnosis and subsequent treatment could be favorable (d-amphetamine in WIS) or unfavorable (atomoxetine and d-amphetamine in WKY) in misdiagnosed individuals.

Treatment and Prevention of Cocaine Abuse

Interestingly, amphetamines have shown efficacy in the treatment of cocaine use disorders. In outbred rats with cocaine self-administration experience, d-amphetamine produces downward shifts in PR cocaine dose-response functions (Chiodo et al, 2008; Zimmer et al, 2014), and reverses cocaine-induced deficits in DAT expression and function (Ferris et al, 2015). In non-human primates, d-amphetamine also reduces cocaine self-administration under PR and second-order schedules of reinforcement, and increases choice for food over cocaine (Czoty et al, 2010; Grabowski et al, 2004; Negus, 2003; Negus & Mello, 2003a, b). Moreover, in cocaine-abusing subjects, moderate doses of amphetamine reduce cocaine intake and craving, and improve treatment retention (Grabowski et al, 2001, 2004; Mooney et al, 2009). In individuals with ADHD and comorbid cocaine use disorder, amphetamine is effective both in reducing cocaine use and in improving ADHD symptoms (Levin et al, 2015). In contrast, methylphenidate is not effective in stimulant-dependent ADHD patients, possibly because of limited efficacy at DAT due to cocaine's long-term effects (Crunelle et al, 2013; Grabowski et al, 2004; Konstenius et al, 2010).

Many other treatments for cocaine use disorders have been investigated, including the administration of other DAT, NET, and SERT inhibitors and monoamine agonists, as well as monoamine antagonists and anti-cocaine vaccines (Grabowski et al, 2004; Howell & Negus, 2014; Kinsey et al, 2010). However, all have been met with limited efficacy. There is currently no "cure" for cocaine use disorder. Recently, preventative measures, particularly for adolescents, have received attention as potentially more effective ways to reduce the prevalence of cocaine use disorder (e.g., Stanis & Andersen, 2014). The current work is the first to demonstrate that adolescent treatment with d-amphetamine may reduce later cocaine abuse risk in outbred rats. Therefore, d-amphetamine treatment may be a viable preventative measure in at-risk teenage populations. The understanding of the neural mechanisms contributing to this protective effect, set forth in this dissertation, may lead to other targeted leads in the treatment of ADHD and prevention of cocaine abuse.

Table 1: Effects of ADHD Medications on Prefrontal Cortex Function in Adolescence and Cocaine Abuse Risk in Adulthood

Medication	PFC Function in Adolescence			Cocaine Abuse Risk in Adulthood					
	Intial Set	Set Shift	Reversal Learning	Acquisition	FR1 Dose-Response	PR Dose-Response	Maintenance	Extinction	Cue-Induced Reinstatement
SHR									
Methylphenidate	Improves[1]	-	-	Increases[2]	Increases[2]	Increases[2]	Increases[3]	-	-
Atomoxetine	Worsens[1]	Improves[1]	-	-	-	-	-	Increases[3]*	Decreases[3]*
d-Amphetamine	-	Improves*	-	Decreases*	-	-	-	-	Decreases*
WIS									
Methylphenidate	Worsens[1]	-	-	Decreases[2]	-	-	-	-	-
Atomoxetine	Improves[1]	-	-	-	-	-	-	-	-
d-Amphetamine	-	-	-	Decreases*	Decreases*	Decreases*	-	-	-
WKY									
Methylphenidate	-	-	-	-	-	-	-	-	-
Atomoxetine	Improves[1]	-	Worsens[1]	Increases[4]*	-	-	-	-	-
d-Amphetamine	-	-	-	Increases*	-	-	-	-	-

[1]Harvey et al, 2013; [2]Harvey et al, 2011; [3]Jordan et al, 2014; [4]Somkuwar et al, 2013a; *Current Findings

Table 2: Monoamine Transporter Functioning in Prefrontal Cortex of Untreated and Treated Rats

Adolescent Medication	SHR			WIS			WKY		
	DAT	NET	SERT	DAT	NET	SERT	DAT	NET	SERT
Untreated	High[1,2]	High[3]	-	-	-	-	Low[9]	High[10]	Low[11]
Methylphenidate	Increases[4]	Decreases[3]	-	Decreases[4]	-	-	Decreases[4]	-	-
Atomoxetine	Decreases[5]	Decreases*	-	Decreases[5]	Decreases[6]	-	Increases[5]	-	Decreases*
d-Amphetamine	Decreases*	Decreases*	Decreases*	Decreases[7,8]	Decreases*	Decreases[12,13]	Increases*	-	Decreases*

[1]Roessner et al, 2010; [2]Watanabe et al, 1997; [3]Somkuwar et al, 2015; [4]Somkuwar et al, 2013b; [5]Somkuwar et al, 2013a; [6]Udvardi et al, 2013; [7]Xie & Miller, 2009; [8]Ferris et al, 2015; [9]Jiao et al, 2003; [10]Tenjani-Butt et al, 1994; [11]Scholl et al, 2010; [12]Haughey et al, 2000; [13]Kokoshka et al, 1998; *Current Proposal

Table 3: Proposed Role of Monoamine Tranporters in Behavior

Monoamine Tranporter	Level of Function	Behavioral Flexibility in Adolescence	Cocaine Abuse Risk in Adulthood
DAT	High	-	Increases
	Low	Improves	Decreases
NET	High	-	-
	Low	Improves	Decreases
SERT	High	-	-
	Low	-	Increases

Future Directions

The results of the current work raise numerous questions for future investigation, particularly regarding the neural mechanisms underlying the effects of atomoxetine and d-amphetamine in SHR, WIS, and WKY. Another important direction for future research is to investigate the long-term effects of ADHD medications in females with ADHD. Women with ADHD may have a higher risk of substance use than men with ADHD (Dalsgaard et al, 2014). Moreover, women tend to progress from recreational drug use to drug dependence faster than men, and have higher relapse rates (Bobzean et al, 2014). Although ADHD is more common in males during childhood, by adulthood the ratio of males to females diagnosed with ADHD is nearly 1:1 (Barkley et al, 2011; Collingwood, 2010). This may be due to higher rates of self-referral by women in adulthood, or because girls are more likely to exhibit the inattentive subtype of ADHD and remain undiagnosed until adolescence or adulthood (Collingwood, 2010). Women with ADHD tend to exhibit more perseverative and internalizing symptoms than men, while men exhibit more

impulsive, hyperactive, and motivational symptoms (Collingwood, 2010; Nussbaum et al, 2012). Women with ADHD are also more likely to develop comorbid anxiety and depressive conditions than men (Collingwood, 2010), and more variable responses to ADHD medication (Nussbaum et al, 2012).

Despite these observations, the effects of stimulant and non-stimulant ADHD medications on cognition and cocaine abuse risk in females are not well known. The SHR may be a valuable starting point in advancing understanding of ADHD comorbidities in females. Similar to the gender differences observed in individuals with ADHD, female SHR exhibit more inattentive and perseverative traits, whereas male SHR exhibit more hyperactivity and impulsivity (Berger & Sagvolden, 1998). Consistent with suggestions that woman with ADHD have higher substance use risk than men, female SHR showed greater sensitization to cocaine and exhibited higher ethanol intake than male SHR (Cailhol & Mormède, 1999; Vendruscolo et al, 2009). In addition, female SHR were more vulnerable to the effects of adolescent methylphenidate treatment on increasing ethanol intake than male SHR (Vendruscolo et al, 2008). Future studies can therefore begin to address the effects of ADHD medication in females with ADHD using the SHR model.

Limitations

There are limitations to every animal model of human disease, and the SHR has not been exempt from criticism. For example, while some studies have observed hyperactivity in the SHR during open field tests, others have shown that SHR

hyperactivity is dependent upon specific task parameters and the control strains that are employed (Ferguson & Cada, 2003; Sagvolden et al, 1992; van den Bergh et al, 2006; Wickens et al, 2011). Notably, the open field test may have little relevance to ADHD, as locomotor activity of children in a playroom did not correlate with hyperactivity ratings or diagnosis (Routh & Schroeder, 1976; Schroeder et al, 1980; Wickens et al, 2011). Other work has questioned the predictive validity of the SHR model (van den Bergh et al, 2006; Wickens et al, 2011). One study found that oral methylphenidate (0.1, 1.0, and 10 mg/kg) did not reduce hyperactivity or impulsivity in the SHR (van den Bergh et al, 2006). However, in this latter study methylphenidate was administered one hour prior to behavioral testing (van den Bergh et al, 2006). Previous work has shown that monoaminergic responses to oral methylphenidate (1.0, 2.5, and 5.0 mg/kg) peak approximately 30 minutes after administration (Kuczenski & Segal, 2002). Thus, monoamine levels may have already peaked if testing occurs 60 minutes after methylphenidate treatment, potentially obscuring any methylphenidate-induced improvements in behavior. Another study found that 3 mg/kg i.p. methylphenidate did not reduce impulsivity in SHR (Bizot et al, 2007). However, this i.p. dose of methylphenidate exceeds clinical relevance, producing elevations in nucleus accumbens dopamine that may be more reminiscent of a drug of abuse (Kuczenski & Segal, 2001). Studies utilizing clinically relevant dosing and treatment regimens (e.g., 1.5 mg/kg orally, 30 minutes prior to behavioral testing) have shown that methylphenidate improves cognitive deficits in the SHR (Harvey et al, 2011; 2013; Kantak et al, 2008). Therefore, rather than questioning the predictive validity of the SHR, together these findings

highlight the importance of experimental designs that are clinically relevant to ADHD, particularly when evaluating ADHD medications.

There are limitations to the current experiments that are worthy of separate comment. For example, a small number of rats expired prior to completing all phases of the experiments within each Aim. Importantly, in all cases death was not caused by experimental factors, but rather to illness ($n = 15$), accidental overdose related to equipment malfunction ($n = 4$), or malfunction of temperature controls in the animal facility ($n = 2$). However, because experimental phases were not directly compared in the statistical analyses (e.g., cocaine intake in Experiment 2 was not compared to cocaine intake in Experiment 1), rat loss had a minimal impact on the current results and their interpretation.

Another limitation of these experiments is that some trend-level ANOVA effects (e.g., $p \leq 0.07$) are reported, e.g., following strain X treatment interactions. However, in cases where trends are reported, post-hoc tests are statistically significant ($p \leq 0.05$), suggesting that these behavioral differences are reliable. One final notable limitation of the current experiments is the use of single doses of atomoxetine and d-amphetamine in the adolescent treatment regimens. Although assessment of multiple of doses may help to elucidate potential dose-related effects of these ADHD medications on cocaine abuse risk, the doses employed in the current work were carefully selected to be clinically relevant using evidence from prior studies. For example, the 0.3 mg/kg atomoxetine dose was chosen because this dose primarily increases extracellular norepinephrine, and to a lesser extent dopamine, in PFC (Bymaster et al, 2002). Higher doses (e.g., 3 mg/kg

atomoxetine), in contrast, have greater effects on extracellular dopamine (Bymaster et al, 2002), which may lead to non-specific behavioral effects. Moreover, the 0.3 mg/kg dose of atomoxetine has been shown to improve behavioral flexibility in adolescent SHR (Harvey et al, 2013). With respect to d-amphetamine, when administered s.c., 0.5 mg/kg amphetamine produces plasma drug concentrations of 84 ± 4 ng/mL in the rat (Heijtz et al, 2003). In patients with ADHD, d-amphetamine may be prescribed at doses ranging from 0.2 - 0.6 mg/kg/day, which produce peak plasma levels ranging from 64 - 84 ng/mL (Brown et al, 1979; Bymaster et al, 2002). Thus, doses higher than 0.5 mg/kg d-amphetamine may produce plasma concentrations that exceed clinical relevance (Heijtz et al, 2003). Although the pharmacokinetcs of s.c. and i.p. routes of administration (the latter of which was used in the current studies) may differ, both routes produce similar plasma levels and time-concentration curves following low-dose psychostimulant administration (Baumann et al, 2009). Moreover, in the rat 0.5 mg/kg d-amphetamine (s.c. or i.p.) does not induce locomotor activation, and increases dopamine and norepinephrine activity as well as dendritic growth in PFC (Brown et al, 1979; Heitjz et al, 2003; Labonte et al, 2011). Because 0.5 mg/kg d-amphetamine did not alleviate all ADHD-related deficits in adolescent SHR, and produced modest protective affects against cocaine abuse risk in adult SHR in the current work, it seems unlikely that investigations of lower doses of d-amphetamine would produce significant behavioral findings. Rather, these observations indicate that the current work employs doses that are representative of and relevant to those used in clinical settings. Nonetheless, future

research may benefit from investigation of the effects of adolescent treatment with alternative doses of atomoxetine and d-amphetamine.

Conclusions

Questions regarding the long-term effects of adolescent ADHD medication on later substance use disorders, and particularly cocaine abuse risk, have been historically difficult to examine in a systematic manner. Preclinical models have promise in addressing gaps in the clinical literature. In particular, the SHR exhibits behavioral and cognitive deficits, as well as neurochemical and genetic differences, reflecting those observed in ADHD. The results of this dissertation, together with previous work, indicate that the SHR has important heuristic value in modeling aspects of cognitive dysfunction, cocaine abuse risk, and cocaine cue reactivity. Moreover, the SHR is an important model for assessing the neurobiology underlying the ADHD phenotype, and for evaluating pharmacotherapeutics for teenagers with ADHD.

Illicit drug use costs the United States over $193 billion each year (NIDA, 2015). Nearly two-thirds of treatment-seeking drug users report sampling drugs before age 20 (SAMHSA, 2002; Stanis & Andersen, 2014). Moreover, initiating drug use before age 14 is associated with four times the likelihood of becoming drug dependent in adulthood (SAMHSA, 2006; Stanis & Andersen, 2014). With approximately 1,600 teens and adults initiating cocaine use each day (SAMHSA, 2013), it becomes critically important to reduce the risk of cocaine abuse, particularly in vulnerable populations. This dissertation

presents novel findings, using an animal model, on the potential consequences of ADHD medication in humans. Specifically, atomoxetine and d-amphetamine may be safer medications than the more commonly prescribed methylphenidate for newly diagnosed teenagers with ADHD. Application of the current preclinical findings to a clinical setting could effectively minimize the risk of SUD in an estimated ~700,000 teenagers with ADHD, who may be receiving medication for the first time (NSCH Database, 2011). Moreover, applying an understanding of the long-term neurochemical effects of drugs of the same class, such as stimulants, to a clinical setting could lead to more appropriate treatments for ADHD.

BIBLIOGRAPHY

Aase, H, Sagvolden, T (2006) Infrequent, but not frequent, reinforcers produce more variable responding and deficient sustained attention in young children with attention-deficit/hyperactivity disorder (ADHD). Journal of Child Psychology and Psychiatry 47: 457-471.

Achat-Mendes, C, Nic Dhonnchadha, BÁ, Platt, DM, Kantak, KM, Spealman, RD (2012) Glycine transporter-1 inhibition preceding extinction training inhibits reacquisition of cocaine seeking. Neuropsychopharmacology 37: 2837-2845.

Adler, LA, Liebowitz, M, Kronenberger, W, Qiao, M, Rubin, R, Hollandbeck, M, Deldar, A, et al (2009) Atomoxetine treatment in adults with attention-deficit/hyperactivity disorder and comorbid social anxiety disorder. Depression and Anxiety 26: 212-221.

Adriani, W, Caprioli, A, Granstrem, O, Carli, M, Laviola, G (2003) The spontaneously hypertensive-rat as an animal model of ADHD: Evidence for impulsive and non-impulsive subpopulations. Neuroscience and Biobehavioral Reviews 27: 639-651.

Adriani, W, Leo, D, Greco, D, Rea, M, di Porzio, U, Laviola, G, Perrone-Capano, C (2006) Methylphenidate administration to adolescent rats determines plastic changes on reward-related behavior and striatal gene expression. Neuropsychopharmacology 31: 1946-1956.

American Academy of Pediatrics Committee on Children With Disabilities and Committee on Drugs (1996) Medication for children with attentional disorders. Pediatrics 98: 301-304.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (5th ed). Arlington, VA: American Psychiatric Publishing.

Andersen, SL (2005) Stimulants and the developing brain. Trends in Pharmacological Sciences 26: 237-243.

Andersen, SL, Arvanitogiannis, A, Pliakas, AM, LeBlanc, C, Carlezon, WA (2001) Altered responsiveness to cocaine in rats exposed to methylphenidate during development. Nature Neuroscience 5: 13-14.

Anderson, SM, Bari, AA, Pierce, RC (2003) Administration of the D1-like dopamine receptor antagonist SCH23390 into the medial nucleus accumbens shell attenuates cocaine priminig-induced reinstatement of drug-seeking in rats. Psychopharmacology (Berlin) 168: 132-138.

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

Arnold, LE, Kirilcuk, V, Corson, SA, Corson, EOL (1973) Levoamphetamine and dextroamphetamine: differential effect on aggression and hyperkinesis in children and dogs. American Journal of Psychiatry 130: 165–170.

Arnsten, AFT, Dudley, AG (2005) Methylphenidate improves prefrontal cortical cognitive function through α2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. Behavioral and Brain Functions 1: 1-9.

Arnsten, AFT, Pliszka, SR (2011) Catecholamine influences on prefrontal cortical function: Relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacology Biochemistry and Behavior 99: 211-216.

Asherson, P, Brookes, K, Chen, W, Gill, M, Ebstein, RP, Buitelaar, J, Banaschewski, T, et al (2007) Confirmation that a specific haplotype of the dopamine transporter gene is associated with combined-type ADHD. American Journal of Psychiatry 16: 674-677.

Bari, AA, Pierce, RC (2005) D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement. Neuroscience 135: 959-968.

Barkley, RA (1997) Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychological Bulletin 121: 65-94.

Barkley, RA (2011) Men, Women and ADHD. The New York Times. Available at http://consults.blogs.nytimes.com/2011/02/11/men-women-and-a-d-h-d/ Accessed 24 July 2015.

Baskin, BM, Dwoskin, LP, Kantak, KM (2015) Methylphenidate treatment beyond adolescence maintains increased cocaine self-administration in the spontaneously hypertensive rat model of attention deficit/hyperactivity disorder. Pharmacology Biochemistry and Behavior 131: 51-56.

Baumann, MH, Zolkowska, D, Kim, I, Scheidweiler, KB, Rothman, RB, Huestis, MA (2009) Effects of dose and route of administration on pharmacokinetics of (±)-3,4-methylenedioxymethamphetamine in the rat. Drug Metabolism and Disposition 37: 2163-2170.

Bayless, DW, Perez, MC, Daniel, JM (2015) Comparison of the validity of the use of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder in males and females. Behavioral Brain Research 286: 85-92.

Berger, DF, Sagvolden, T (1998) Sex differences in operant discrimination behaviour in an animal model of attention-deficit hyperactivity disorder. Behavioral Brain Research 94: 73-82.

Berglund, EC, Makos, MA, Keighron, JD, Phan, N, Heien, ML, Ewing, AG (2013) Oral administration of methylphenidate blocks the effect of cocaine on uptake at the Drosophila dopamine transporter. American Chemical Society Chemical Neuroscience 4: 566-574.

Biederman, J, Monuteaux, MC, Mick, E, Wilens, TE, Fontanella, JA, Poetzl, KM, et al (2006) Is cigarette smoking a gateway to alcohol and illicit drug use disorders? A study of youths with and without attention deficit hyperactivity disorder. Biological Psychiatry 59: 258-264.

Biederman, J, Petty, CR, Evans, M, Small, J, Faraone, SV (2010) How persistent is ADHD? A controlled 10-year follow-up study of boys with ADHD. Psychiatry Research 177: 299-304.

Birrell, JM, Brown, VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. Journal of Neuroscience 20: 4320-4324.

Bizot, JC, Chenault, N, Houzé, B, Herpin, A, David, S, Pothion, S, Trovero, F (2007) Methylphenidate reduces impulsive behaviour in juvenile Wistar rats, but not in adult Wistar, SHR and WKY rats. Psychopharmacology (Berlin) 193: 215-223.

Bobzean, SAM, DeNobrega, AK, Perrotti, LI (2014) Sex differences in the neurobiology of drug addiction. Experimental Neurology 259: 64-74.

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

Booth, JR, Burman, DD, Meyer, JR Lei, Z, Trommer, BL, Davenport, ND, Wei, Li, et al (2005) Larger deficits in brain networks for response inhibition than for visual selective attention in attention deficit hyperactivity disorder (ADHD). Journal of Child Psychology and Psychiatry 46: 94-111.

Brackney, RJ, Cheung, THC, Herbst, K, Hill, JC, Sanabria, F (2012) Extinction learning deficit in a rodent model of attention-deficit hyperactivity disorder. Behavioral and Brain Functions 8: 59-68

Brandon, CL, Marinelli, M, Baker, LK, White, FJ (2001) Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 25: 651-661.

Brenhouse, HC, Sonntag, KC, Andersen, SL (2008) Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: Relationship to enhanced motivational salience of drug cues in adolescence. Journal of Neuroscience 28: 2375-2382.

Brown, GL, Hunt, RD, Ebert, MH, Bunney Jr, WE, Kopin, IJ (1979) Plasma levels of d-amphetamine in hyperactive children: Serial behavior and motor responses. Psychopharmacology (Berlin) 62: 133-140.

Buchta, WC, Riegel, AC (2015) Chronic cocaine disrupts mesocortical learning mechanisms. Brain Research. Available 20 February 2015.

Buitelaar, JK, Casas, M, Philipsen, A, Kooij, JJS, Ramos-Quiroga, JA, Dejonckheere, J, van Oene, JC, Schauble, B (2011) Functional improvement and correlations with symptomatic improvement in adults with attention deficit hyperactivity disorder receiving long-acting methylphenidate. Psychological Medicine 42: 195-204.

Burgess, GC, Depue, BE, Ruzic, L, Willcutt, EG, Du, YP, Banich, MT (2010) Attentional Control Activation Relates to Working Memory in Attention-Deficit/Hyperactivity Disorder. Biological Psychiatry 67: 632-640.

Bymaster, FP, Katner, JS, Nelson, DL, Hemrick-Luecke, SK, Threlkeld, PG, Heiligenstein, JH, et al (2002) Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: A potential mechanism for efficacy in Attention Deficit/Hyperactivity Disorder. Neuropsychopharmacology 27: 699-711.

Caihol, S, Mormède, P (1999) Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. Brain Research 842: 200-205.

Cain, RE, Wasserman, MC, Waterhouse, BD, McGaughy, JA (2011) Atomoxetine facilitates attentional set shifting in adolescent rats. Developmental Cognitive Neuroscience 1: 552-559.

Calipari, ES, Ferris, MJ, Zimmer, BA, Robers, DCS, Jones, SR (2013) Temporal Pattern of Cocaine Intake Determines Tolerance vs Sensitization of Cocaine Effects at the Dopamine Transporter. Neuropsychopharmacology 38: 2385-2392.

Cao, J, Lotfipour, S, Loughlin, SE, Leslie, FM (2007) Adolescent maturation of cocaine-sensitive neural mechanisms. Neuropsychopharmacology 32: 2279-2289.

Carlezon, WA, Mague, SD, Andersen, SL (2003) Enduring behavioral effects of early exposure to methylphenidate in rats. Biological Psychiatry 54: 1330-1337.

Carrey, N, MacMaster, FP, Fogel, J, Sparkes, S, Waschbusch, D, Sullivan, S, Schmidt, M (2003) Metabolite changes resulting from treatment in children with ADHD: a 1H-MRS study. Clinical Neuropharmacology 26: 218-221.

Castle, L, Aubert, RE, Verbrugge, RR, Khalid, M, Epstein, RS (2007) Trends in Medication Treatment for ADHD. Journal of Attention Disorders 10: 335-342.

Chamberlain, SR, Hampshire, A, Müller, U, Rubia, K, del Campo, N, Craig, K, Regenthal, R, et al (2009) Atomoxetine modulates right inferior frontal activation during inhibitory control: A pharmacological functional magnetic resonance imaging study. Biological Psychiatry 65: 550-555.

Chen, BT, Bowers, MS, Martin, M, Hopf, FW, Guillory, AM, Carelli, RM, Bonci, A (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron 59: 288-297.

Chiodo KA, Läck CM, Roberts DC (2008) Cocaine self-administration reinforced on a progressive ratio schedule decreases with continuous D-amphetamine treatment in rats. Psychopharmacology (Berlin) 200: 465-473.

Ciccocioppo, R, Paolo Sanna, P, Weiss, F (2001) Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: Reversal by D1 antagonists. Proc Nat Acad Sci USA 98: 1976-1981.

Collingwood, J (2010) ADHD and Gender. Psychology Central. Available at http://psychcentral.com/lib/adhd-and-gender/ Accessed 24 July 2015.

Colzato, LS, Huizinga, M, Hommel, B (2009) Recreational cocaine polydrug use impairs cognitive flexibility but not working memory. Psychopharmacology 207: 225-234.

Cominski, TP, Jiao, X, Catuzzi, JE, Stewart, AL, Pang, KC (2014) The role of the hippocampus in avoidance learning and anxiety vulnerability. Frontiers in Behavioral Neuroscience 8: 273.

Courvoisie, H, Hooper, SR, Fine, C, Kwock, L, Castillo, M (2004) Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: Preliminary findings. Journal of Neuropsychiatry and Clinical Neuroscience 16: 63-69.

Crawford, CA, Baella, SA, Farley, CM, Herbert, MS, Horn, LR, Campbell, RH, Zavala, AR (2011) Early methylphenidate exposure enhances cocaine self-administration but not cocaine-induced conditioned place preference in young adult rats. Psychopharmacology 213: 43-52.

Crofts, HS, Dalley, JW, Collins, P, Van Denderen, JC, Everitt, BJ, Robbins, TW, et al (2001) Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cerebral Cortex 11: 1015-1026.

Crunelle CL, van den Brink W, Veltman DJ, van Emmerik-van Oortmerssen K, Dom G, Schoevers RA, Booij J (2013) Low dopamine transporter occupancy by methylphenidate as a possible reason for reduced treatment effectiveness in ADHD patients with cocaine dependence. European Neuropsychopharmacology 23: 1714-1723.

Cubillo A, Halari R, Giampietro V, Taylor E, Rubia K (2011) Fronto-striatal underactivation during interference inhibition and attention allocation in grown up children with attention deficit/hyperactivity disorder and persistent symptoms. Psychiatry Research 193: 17-27.

Cubillo, A, Halari, R, Smith, A, Taylor, E, Rubia, K (2012) A review of fronto-striatal and fronto-cortical brain abnormalities in children and adults with attention deficit hyperactivity disorder (ADHD) and new evidence for dysfunction in adults with ADHD during motivation and attention. Cortex 48: 194-215.

Cubillo A, Smith AB, Barrett N, Giampietro V, Brammer M, Simmons A, Rubia K (2014) Drug-specific laterality effects on frontal lobe activation of atomoxetine and methylphenidate in attention deficit hyperactivity disorder boys during working memory. Psychology Medicine 44: 633-646.

Currie, J, Stabile, M, Jones, LE (2014) Do stimulant medications improve educational and behavioral outcomes for children with ADHD? Journal of Health Economics 37: 58-69.

Czoty PW, Martelle JL, Nader MA (2010) Effects of chronic d-amphetamine administration on the reinforcing strength of cocaine in rhesus monkeys. Psychopharmacology (Berlin) 209: 375-382.

Dalsgaard, S, Mortensen, PB, Frydenberg, M, Thomsen, PH (2014) ADHD, stimulant treatment in childhood and subsequent substance abuse in adulthood - a naturalistic long-term follow-up study. Addictive Behaviors 39: 325-328.

Degenhardt, L, Chiu, WT, Sampson, N, Kessler, RC, Anthony, JC, Angermeyer, M, et al (2008) Toward a global view of alcohol, tobacco, cannabis, and cocaine use: Findings from the WHO World Health Mental Surveys. PLoS Medicine 5: e141.

De La Garza II, R (2005) Wistar Kyoto rats exhibit reduced sucrose pellet reinforcement behavior and intravenous nicotine self-administration. Pharmacology Biochemistry and Behavior 85: 330-337.

De La Garza II, R, Mahoney III, JJ (2004) A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. Brain Research 1021: 209-218.

dela Peña, I, Gonzales, EL, de la Peña, JB, Kim, B-N, Han, DH, Shin, CY, Cheong, JH (2015) Individual differences in novelty-seeking behavior in spontaneously hypertensive rats: Enhanced sensitivity to the reinforcing effect of methylphenidate in the high novelty-preferring subpopulation. Journal of Neuroscience Methods. Available 26 August 2014.

dela Peña, I, Lee, JC, Lee, HL, Woo, TS, Lee, HC, Sohn, AR, Cheong, JH (2012) Differential behavioral responses of the spontaneously hypertensive rat to methylphenidate and methamphetamine: Lack of a rewarding effect of repeated methylphenidate treatment. Neuroscience Letters 514: 189-193.

de los Cobos, JP, Siñol, N, Puerta, C, Cantillano, V, Zurita, CL, Trujols, J (2011) Features and prevalence of patients with probable adult Attention Deficit Hyperactivity Disorder who request treatment for cocaine use disorders. Psychiatry Research 185: 205-210.

Depue, BE, Burgess, GC, Willcutt, EG, Ruzic, L, Banich, MT (2010) Inhibitory control of memory retrieval and motor processing associated with the right lateral prefrontal cortex: Evidence from deficits in individuals with ADHD. Neuropsychologia 48: 3909-3917.

Dick, AS (2012) Sources of Cognitive Inflexibility in Set-Shifting Tasks: Insights Into Developmental Theories From Adult Data. Journal of Cognition and Development 13: 82-110.

Ding, YS, Naganawa, M, Gallezot, JD, Nabulsi, N, Lin, SF, Ropchan, J, et al (2014) Clinical doses of atomoxetine significantly occupy both norepinephrine and serotonin transports: Implications on treatment of depression and ADHD. Neuroimage 86: 164-171.

Di Pietro, NC, Mashhoon, Y, Heaney, C, Yager, LM, Kantak, KM (2008) Role of dopamine D1 receptors in the prefrontal dorsal agranular insular cortex in mediating cocaine self-administration in rats. Psychopharmacology 200: 81-91.

Donfrancesco, R, Di Trani, M, Porfirio, MC, Giana, G, Miano, S, Andriola, E (2015) Might the temperament be a bias in clinical study on attention-deficit hyperactivity

disorder (ADHD)?: Novelty seeking dimension as a core feature of ADHD. Psychiatry Research 227: 333-338.

De Vries, TJ, Cools, AR, Shippenberg, TS (1998) Infusion of a D-1 receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitization. Neuroreport 9: 1763-1768.

Durston, S, Fossella, JA, Casey, BJ, Hulshoff, Pol, HE, Galvan, A, Schnack, HG, et al (2005) Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Molecular Psychiatry 10: 678-685.

Easton, N, Steward, C, Marshall, F, Fone, K, Marsden, C (2007) Effects of amphetamine isomers, methylphenidate, and atomoxetine on synaptosomal and synaptic vesicle accumulation and release of dopamine and noreadrenaline in vitro in the rat brain. Neuropharmacology 52: 405-414.

Economidou, D, Dalley, JW, Everitt, BJ (2011) Selective norepinephrine reuptake inhibition by atomoxetine prevents cue-induced heroin and cocaine seeking. Biological Psychiatry 69: 266-274.

Faraone, SV, Biederman, J, Spencer, TJ, Aleardi, M (2006) Comparing the efficacy of medications for ADHD using meta-analysis. Medscape General Medicine 8: 4.

Faraone, SV, Buitelaar, J (2010) Comparing the efficacy of stimulants for ADHD in children and adolescents using meta-analysis. European Child and Adolescent Psychiatry 19: 353-364.

Faraone, SV, Perlis, RH, Doyle, AE, Smoller, JW, Goralnick, JJ, Holmgren, MA, Sklar, P (2005) Molecular genetics of attention-deficit/hyperactivity disorder. Biological Psychiatry 57: 1313-1323.

Faraone, SV, Spencer, TJ, Montano, B, Biederman, J (2004) Attention-Deficit/Hyperactivity Disorder in adults: A survey of current practice in psychiatry and primary care. JAMA Internal Medicine 164: 1221-1226.

Faraone, SV, Wilens, TE, Petty, C, Antshel, K, Spencer, T, Biederman, J (2007) Substance use among ADHD adults: implications of late onset and subthreshold diagnoses. The American Journal on Addiction 16: 24-34.

Ferguson, SA, Cada, AM (2003) A longitudinal study of short- and long-term activity levels in male and female spontaneously hypertensive, Wistar-Kyoto, and Sprague-Dawley rats. Behavioral Neuroscience 117: 271-282.

Ferris, MJ, Calipari, ES, Rose, JH, Siciliano, CA, Sun, H, Chen, R, Jones, SR (2015) A single amphetamine infusion reverses deficits in dopamine nerve-terminal function caused by a history of cocaine self-administration. Neuropsychopharmacology 40: 1826-1836.

Floresco, SB (2013) Dopamine and behavioral flexibility: Shifting from an "inverted-U" toward a family of functions. Frontiers in Neuroscience 7: 1-12.

Floresco, SB, Block, AE, Tse, MTL (2008) Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behavioural Brain Research 190: 85-96.

Floresco, SB, Magyar, O, Ghods-Sharifi, S, Vexelman, C, Tse, MTL (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. Neuropsychopharmacology 31: 297-309.

Fusar-Poli P, Rubia K, Rossi G, Sartori G, Balottin U (2012) Striatal dopamine transporter alterations in ADHD: Pathophysiology or adaptation to psychostimulants? A meta-analysis. The American Journal of Psychiatry 169: 264-272.

Franke, B, Vasquez, AA, Johansson, S, Hoogman, M, Romanos, J, Boreatti-Hümmer, A, et al (2010) Multicenter analysis of the SLC6A3/DAT1 VNTR haplotype in persistent ADHD suggests differential involvement of the gene in childhood and persistent ADHD. Neuropsychopharmacology 35: 656-664.

Gamo, NJ, Wang, M, Arnsten, AFT (2010) Methylphenidate and atomoxetine enhance prefrontal function through α2-adrenergic and dopamine D1 receptors. Journal of the American Academy of Child Adolescent Psychiatry 49: 1011-1023.

Gatley, SJ, Pan, D, Chen, R, Chaturvedi, G, Ding, Y-S (1996) Affinities of methylphenidate derivatives for dopamine, norepinephrine, and serotonin transporters. Life Sciences 58: 231-239.

Geller, D, Donnelly, C, Lopez, F, Rubin, R, Newcorn, J, Sutton, V, Bakken, R, et al (2007) Atomoxetine treatment for pediatric patients with attention-deficit/hyperactivity disorder with comorbid anxiety disorder. Journal of the Academy of Child and Adolescent Psychiatry 46: 1119-1127.

Ghods-Sharifi, S, Haluk, DM, Floresco, SB (2008) Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. Neurobiology of Learning and Memory 89: 567-573.

Goez, H, Back-Bennet, O, Zelnik, N (2007) Differential stimulant response on attention in children with comorbid anxiety and oppositional defiant disorder. Journal of Child Neurology 22: 538-542.

Gudjonsson, GH, Sigurdsson, JF, Sigfusdottir, ID, Young, S (2012) An epidemiological study of ADHD symptoms among young persons and the relationship with cigarette smoking, alcohol consumption, and illicit drug use. Journal of Child Psychology and Psychiatry 53: 304-312.

Guindalini, C, Howard, M, Haddley, K, Laranjeira, R, Collier, D, Ammar, N, et al (2006) A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. Proc Nat Acad Sci USA 103: 4552-4557.

Grabowski, J, Rhoades, H, Schmitz, J, Stotts, A, Daruzska, LA, Creson, D, Moeller, FG (2001) Dextroamphetamine for cocaine-dependence treatment: A double-blind randomized clinical trial. Journal of Clinical Psychopharmacology 21: 522-526.

Grabowski, J, Shearer, J, Merrill, J, Negus, SS (2004) Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. Addictive Behaviors 29: 439-464.

Grace, AA (2001) Psychostimulant actions on dopamine and limbic system function: Relevance to the pathophysiology and treatment of ADHD. In: Solanto, MV, Arnsten, AFT, Castellanos, FX (Eds). Stimulant Drugs and ADHD: Basic and Clinical Neuroscience. Oxford University Press: London, United Kingdom pp 134-157.

Greenbaum, D, Colangelo, C, Williams, K, Gerstein, M (2003) Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biology 4: 117.

Harvey, RC, Jordan, CJ, Tassin, DH, Moody, KR, Dwoskin, LP, Kantak, KM (2013) Performance on a strategy set shifting task during adolescence in a genetic model of attention deficit/hyperactivity disorder: Methylphenidate vs atomoxetine treatments. Behavioural Brain Research 244: 38-47.

Harvey, RC, Sen, S, Deaciuc, A, Dwoskin, LP, Kantak, KM (2011) Methylphenidate treatment in adolescent rats with an attention deficit/hyperactivity disorder phenotype: Cocaine addiction vulnerability and dopamine transporter function. Neuropsychopharmacology 36: 837-847.

Haughey, HM, Fleckenstein, AE, Metzger, RR, Hanson, GR (2000) The effects of methamphetamine on serotonin transporter activity: role of dopamine and hyperthermia. Journal of Neurochemistry 75: 1608-1617.

Heal, DJ, Smith, SL, Findling, RL (2012) ADHD: Current and future therapeutics. Current Topics in Behavioral Neuroscience 9: 361-390.

Heal, DJ, Smith, SL, Gosden, J, Nutt, DJ (2013) Amphetamine, past and present - a pharmacological and clinical perspective. Journal of Psychopharmacology 27: 479-496.

Heijtz, RD, Kolb, B, Forssberg, H (2003) Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? European Journal of Neuroscience 18: 3394-3399.

Henry, DJ, White, FJ (1991) Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within the rat nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics 258: 882-890.

Hesse, S, Ballaschke, O, Barthel, H, Sabri, O (2009) Dopamine transporter imaging in adult patients with Attention-Deficit/Hyperactivity Disorder Psychiatry Research. Neuroimaging 171: 120-128.

Hester, R, Garavan, H (2004) Executive dysfunction in cocaine addiction: Evidence for discordant frontal, cingulate, and cerebellar activity. Journal of Neuroscience 24: 11017-11022.

Hill, JC, Herbst, K, Sanabria, F (2012) Characterizing operant hyperactivity in the spontaneously hypertensive rat. Behavioral and Brain Functions 8: 5.

Hohmann, S, Hohm, E, Treutlein, J, Blomeyer, D, Jennen-Steinmetz, C, Schmidt, MH, et al (2015) Association of norepinephrine transporter (NET, SLC6A2) genotype with ADHD-related phenotypes: findings of a longitudinal study from birth to adolescence. Psychiatry Research 226: 425-433.

Homberg, JR, De Boer, SF, Raasø, HS, Olivier, JD, Verheul, M, Ronken, E, et al (2008) Adaptations in pre- and postsynaptic 5-HT1A receptor function and cocaine supersensitivity in serotonin transporter knockout rats. Psychopharmacology (Berlin) 200: 367-380.

Hoogman, M, Onnink, M, Cools, R, Aarts, E, Kan, C (2012) The dopamine transporter haplotype and reward-related striatal responses in adult ADHD. European Neuropsychopharmacology: 23: 469-478.

Hooks, MS, Jones, GH, Smith, AD, Neill, DB, Justice, JB Jr (1991)
Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9: 121-128.

Hooks, MS, Juncos, JL, Justice, JB Jr, Meiergerd, SM, Povlock, SL, Schenk, JO, Kalivas, PW (1994) Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. Journal of Neuroscience 14: 6144-6152.

Howell, LL, Negus, SS (2014) Monoamine transporter inhibitors and substrates as treatments for stimulant abuse. Advances in Pharmacology 69: 129-176.

Humphreys, KL, Eng, T, Lee, SS (2013) Stimulant medication and substance use outcomes: A meta-analysis. JAMA Psychiatry 70: 740-749.

Ikemoto, S, Glazier, BS, Murphy, JM, McBride, WJ (1997) Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. Journal of Neuroscience 17: 8580-8587.

Janak, PH, Bowers, MS, Corbit, LH (2012) Compound stimulus presentation and the norepinephrine reuptake inhibitory atomoxetine enhance long-term extinction of cocaine-seeking behavior. Neuropsychopharmacology 37: 975-985.

Jasinska, AJ, Stein, EA, Kaiser, J, Naumer, MJ, Yalachkov, Y (2014) Factors modulating neural reactivity to drug cues in addiction: A survey of human neuroimaging studies. Neuroscience and Biobehavioral Reviews 38: 1-16.

Jiao, X, Pare, WP, Tejani-Butt, SM (2003) Strain differences in the distribution of dopamine transporter sites in rat brain. Progress in Neuropsychopharmacology and Biological Psychiatry 27: 913- 919.

Johansen, EB, Aase, H, Meyer, A, Sagvolden, T (2002) Attention-deficit/hyperactivity disorder (ADHD) behaviour explained by dysfunctioning reinforcement and extinction processes. Behavioural Brain Research 130: 37- 45.

Johansen, EB, Sagvolden, T, Kvande, G (2005) Effects of delayed reinforcers on the behavior of an animal model of attention-deficit/hyperactivity disorder (ADHD). Behavioural Brain Research 162: 47-61.

Jordan, CJ, Harvey, RC, Baskin, BB, Dwoskin, LP, Kantak, KM (2014) Cocaine-seeking behavior in a genetic model of attention-deficit/hyperactivity disorder following adolescent methylphenidate or atomoxetine treatments. Drug and Alcohol Dependence 140: 25-32.

Kado, Y, Sanada, S, Yanagihara, M, Ogino, T, Ohno, S, Watanabe, K, et al (2012) Executive function in children with pervasive developmental disorder and attention-deficit/hyperactivity disorder assessed by the Keio version of the Wisconsin card sorting test. Brain Development 34: 354-359.

Kantak, KM, Barlow, N, Tassin, DH, Brisotti, MF, Jordan, CJ (2014) Performance on a strategy set shifting task in rats following adult or adolescent cocaine exposure. Psychopharmacology (Berlin) 231: 4489-4501.

Kantak, KM, Black, Y, Valencia, E, Green-Jordan, K, Eichenbaum, HB (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. Journal of Neuroscience 22: 1126-1136.

Kantak, KM, Mashhoon, Y, Silverman, DN, Janes, AC, Goodrich, CM (2009) Role of the orbitofrontal cortex and dorsal striatum in regulating the dose-related effects of self-administered cocaine. Behavioural Brain Research 201: 128-136.

Kantak, KM, Singh, T, Kerstetter, KA, Dembro, KA, Mutebi, MM, Harvey, RC, et al (2008) Advancing the spontaneous hypertensive rat model of Attention Deficit/Hyperactivity Disorder. Behavioral Neuroscience 122: 340-357.

Kaplan, GB, Heinrichs, SC, Carey, RJ (2011) Treatment of addiction and anxiety using extinction approaches: Neural mechanisms and their treatment implications. Pharmacology Biochemistry and Behavior 97: 619-625.

Kim, Y, Teylan, MA, Baron, M, Sands, A, Nairn, AC, Greengard, P (2009) Methylphenidate-induced dendritic spine formation and DeltaFosB expression in nucleus accumbens. Proc Nat Acad Sci USA 106: 2915-2920.

Kinsey, BM, Kosten, TR, Orson, FM (2010) Active immunotherapy for the treatment of cocaine dependence. Drugs of the Future 35: 301-306.

Kollins, SH, Lane, SD, Shapiro, SK (1997) Experimental analysis of childhood psychopathology: a laboratory matching analysis of the behavior of children diagnosed with attention-deficit hyperactivity disorder (ADHD). The Psychological Record 47: 25-44.

Kofler, MJ, Rapport, MD, Sarver, DE, Raiker, JS, Orban, SA, Friedman, LM, Kolomeyer, EG (2013) Reaction time variability in ADHD: A meta-analytic review of 319 studies. Clinical Psychology Review 33: 795-811.

Kokoshka, JM, Metzger, RR, Wilkins, DG, Gibb, JW, Hanson, GR, Fleckenstein, AE (1998) Methamphetamine treatment rapidly inhibits serotonin, but not glutamate, transporters in rat brain. Brain Research 799: 78-83.

Konstenius, M, Jayaram-Lindström, N, Beck, O, Franck, J (2010) Sustained release methylphenidate for the treatment of ADHD in amphetamine abusers: A pilot study. Drug and Alcohol Dependence 108: 130-133.

Kourrich, S, Rothwell, PE, Klug, JR, Thomas, MJ (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. Journal of Neuroscience 27: 7921-7928.

Kousha, M, Shahrivar, Z, Alaghband-rad, J (2012) Substance use disorder and ADHD: Is ADHD a particularly "specific" risk factor? Journal of Attention Disorders 16: 325-332.

Kosten, TR, Scanley, BE, Tucker, KA, Oliveto, A, Prince, C, Sinha, R, et al (2005) Cue-induced brain activity changes and relapse in cocaine-dependent patients. Neuropsychopharmacology 31: 644-650.

Kratochvil, CJ, Heiligenstein, JH, Dittman, R, Spencer, TJ, Biederman, J, Wernicke, J, et al (2002) Atomoxetine and methylphenidate treatment in children with ADHD: A prospective, randomized, open-label trial. Journal of the American Academy of Child and Adolescent Psychiatry 41: 776-784.

Kuczenski, R, Segal, DS (2002) Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. Journal of Neuroscience 22: 7264-7271.

Kuczenski, R, Segal, DS (2001) Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: Relative roles of dopamine and norepinephrine. Journal of Pharmacology and Experimental Therapeutics 296: 876-883.

Labonte, B, McLaughlin, RJ, Dominguez-Lopez, S, Bambico, FR, Lucchino, I, Ochoa-Sanchez, R, et al (2011) Adolescent amphetamine exposure elicits dose-specific effects on monoaminergic neurotransmission and behaviour in adulthood. International Journal of Neuropsychopharmacology 15: 1319-1330.

Lambert, N (2005) The contribution of childhood ADHD, conduct problems, and stimulant treatment to adolescent and adult tobacco and psychoactive substance abuse. Ethical Human Psychology and Psychiatry 7:197-221.

Lapiz, MD, Morilak, DA (2006) Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. Neuroscience 137: 1039-1049.

Lambert, NM, Hartsough, CS (1998) Prospective study of tobacco smoking and substance dependencies among samples of ADHD and non-ADHD participants. Journal of Learning Disabilities 31: 533-544.

Lee, SS, Humphreys, KL, Flory, K, Liu, R, Glass, K (2011) Prospective association of childhood attention-deficit/hyperactivity disorder (ADHD) and substance use and abuse/dependence: A meta-analytic review. Clinical Psychology Review 31: 328-341.

Levin, ED, Lawrence, SS, Petro, A, Horton, K, Rezvani, AH, Seidler, FJ, et al (2007) Adolescent vs. adult-onset nicotine self-administration in male rats: Duration of effect and differential nicotinic receptor correlates. Neurotoxicolology and Teratology 29: 458-465.

Levin, FR, Mariani, JJ, Specker, S, Mooney, M, Mahony, A, Brooks, DJ (2015) Extended-Release Mixed Amphetamine Salts vs Placebo for Comorbid Adult Attention-Deficit/Hyperactivity Disorder and Cocaine Use Disorder: A Randomized Clinical Trial. JAMA Psychiatry 72: 593-602.

Li, Y, Hu, XT, Berney, TG, Vartanian, AJ, Stine, CD, Wolf, ME, White, FJ (1999) Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. Synapse 24: 169-180.

Li, Q, Lu, G, Antonio, GE, Mak, YT, Rudd, JA, Fan, M, Yew, DT (2007) The usefulness of the spontaneously hypertensive rat to model attention-deficit/hyperactivity disorder (ADHD) may be explained by the differential expression of dopamine-related genes in the brain. Neurochemistry International 50: 848-857.

Lindemann, L, Meyer, CA, Jeanneau, K, Bradaia, A, Ozmen, L, Bluethmann, H, Bettler, B, et al (2008) Trace amine-associated receptor 1 modulates dopaminergic activity. Journal of Pharmacology and Experimental Therapeutics 324: 948-956.

Loh, EA, Roberts, DCS (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology 101: 262-266.

Lüscher, C, Malenka, RC (2011) Drug-evoked synaptic plasticity in addiction: From molecular changes to circuit remodeling. Neuron 69: 650-663.

MacDonald, SWS, Nyberg, L, Bäckman, L (2006) Intra-individual variability in behavior: Links to brain structure, neurotransmission and neuronal activity. Trends in Neuroscience 29: 474-480.

MacMaster, FP, Carrey, N, Sparkes, S, Kusumakar, V (2003) Proton spectroscopy in medication-free pediatric attention-deficit/hyperactivity disorder. Biological Psychiatry 53: 184-187.

Mannuzza, S, Klein, RG, Truong, NL, Moulton, JL, Roizen, ER, Howell, KH, et al (2008) Age of methylphenidate treatment initiation in children with ADHD and later substance abuse: Prospective follow-up into adulthood. American Journal of Psychiatry 165: 604-609.

Martin, BJ, Naughton, BJ, Thirtamara-Rajamani, K, Yoon, DJ, Han, DD, Devries, AC, Gu, HH (2011) Dopamine transporter inhibition is necessary for cocaine-induced increases in dendritic spine density in the nucleus accumbens. Synapse 65: 490-496.

Martins S, Tramontina S, Polanczyk G, Eizirik M, Swanson JM, Rohde LA (2004) Weekend holidays during methylphenidate use in ADHD children: A randomized clinical trial. Journal of Child and Adolescent Psychopharmacology 14: 195-206.

Martinussen, R, Hayden, J, Hogg-Johnson, S, Tannock, R (2005) A meta-analysis of working memory impairments in children with Attention-Deficit/Hyperactivity Disorder. Journal of the American Academy of Child and Adolescent Psychiatry 44: 377-384.

Mash, DC, Pablo, J, Ouyang, Q, Hearn, WL, Izenwasser, S (2002) Dopamine transport function is elevated in cocaine users. Journal of Neurochemistry 81: 292-300.

Mateo, Y, Lack, CM, Morgan, D, Roberts, DC, Jones, SR (2005) Reduced dopamine terminal function and insensitivity to cocaine following cocaine binge self-administration and deprivation. Neuropsychopharmacology 30(8): 1455-1463.

Mattiuz, EL, Ponsler, GD, Barbuch, RJ, Wood, PG, Mullen, JH, Shugert, RL, et al (2003) Disposition and metabolic fate of atomoxetine hydrochloride: Pharmacokinetics, metabolism, and excretion in the Fischer 344 rat and beagle dog. Drug Metabolism and Disposition 31: 88- 97.

McCarthy, S, Asherson, P, Coghill, D, Hollis, C, Murray, M, Potts, L, et al (2009) Attention-deficit hyperactivity disorder: Treatment discontinuation in adolescents and young adults. British Journal of Psychiatry 194: 273-277.

McGaughy, J, Ross, RS, Eichenbaum, H (2008) Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting. Neuroscience 153: 63-71.

McGregor, A, Roberts, DC (1993) Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Research 624: 245-252.

McGregor, A, Roberts, DC (1995) Effect of medial prefrontal cortex injections of SCH 23390 on intravenous cocaine self-administration under both a fixed and progressive ratio schedule of reinforcement. Behavioural Brain Research 67: 75-80.

Meneses, A, Ponce-Lopez, T, Tellez, R, Gonzalez, R, Castillo, C, Gasbarri, A (2011) Effects of d-amphetamine on short- and long-term memory in spontaneously

hypertensive, Wistar-Kyoto, and Sprague-Dawley rats. Behavioural Brain Research 216: 472-476.

Mill, J, Sagvolden, T, Asherson, P, (2005) Sequence analysis of Drd2, Drd4, and Dat1 in SHR and WKY rat strains. Behavioral Brain Functions 1: 24.

Miller, GM (2011) The emerging role of trace amine-associated receptor 1 in the functional regulation of monoamine transporters and dopaminergic activity. Journal of Neurochemistry 116: 164-176.

Miller, EM, Pomerleau, F, Huettl, P, Gerhardt, GA, Glaser, PEA (2014) Aberrant glutamate signaling in the prefrontal cortex and striatum of the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder. Psychopharmacology (Berlin) 231: 3019-3029.

Mitchell, JT, McIntyre, EM, McClernon, FJ, Kollins, SH (2013) Smoking motivation in adults with attention-deficit/hyperactivity disorder using the Wisconsin inventory of smoking dependence motives. Nicotine and Tobacco Research 16: 120-125.

Molina, BSG, Hinshaw, SP, Arnold, LE, Swanson, JM, Pelham, WE, Hechtman, L, et al (2013) Adolescent substance use in the multimodal treatment study of attention-deficit/hyperactivity disorder (ADHD) (MTA) as a function of childhood ADHD, random assignment to childhood treatments, and subsequent medication. Journal of the American Academy of Child and Adolescent Psychiatry 52: 250-263.

Moll, GH, Mehnert, C, Wicker, M, Bock, N, Rothenberger, A, Rüther, E, Huether, G (2000) Age-associated changes in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood. Developmental Brain Research 119: 251-257.

Moon, SJ, Kim, CJ, Lee, YJ, Hong, M, Han, J, Bahn, GH (2014) Effect of atomoxetine on hyperactivity in an animal model of attention-deficit/hyperactivity disorder (ADHD). PLoS ONE 9: e108918.

Mooney, ME, Herin, DV, Schmitz, JM, Moukaddam, N, Green, CE, Grabowski, J (2009) Effects of oral methamphetamine on cocaine use: a randomized, double-blind, placebo-controlled trial. Drug and Alcohol Dependence 101: 34-41.

Morón, JA, Brockington, A, Wise, RA, Rocha, BA, Hope, BT (2002) Dopamine uptake through the norepinephrine transporter in regions with low levels of the dopamine transporter: Evidence from knock-out mouse lines. Journal of Neuroscience 22: 389-395.

MTA Cooperative Group (1999) A 14-month randomized clinical trial of treatment strategies for Attention-Deficit/Hyperactivity Disorder. Archives of General Psychiatry 56: 1073-1086.

Nakagawa, Y, Chin, W, Imai, S (1982) Effects of guanfacine on pre- and postsynaptic alpha-adrenoceptors studied in comparison with those of clonidine. Nihon Yakurigaku Zasshi 79: 431-439.

Narendran, R, Lopresti, BJ, Martinez, D, Mason, NS, Himes, M, May, MA (2012) In vivo evidence for low striatal vesicular monoamine transporter 2 (VMAT2) availability in cocaine abusers. Americal Journal of Psychiatry 169: 55-63.

National Bureau of Economic Research (NBER; 2015) The Effects of Stimulant Medications for ADHD. Available at http://www.nber.org/aginghealth/2014no1/w19105.html Accessed 24 July 2015.

National Institute of Drug Abuse (NIDA; 2015) Trends & statistics: Costs of substance abuse. Available at http://www.drugabuse.gov/related-topics/trends-statistics#costs Accessed 24 July 2015.

National Survey of Children's Health (2011) Child and Adolescent Health Measurement Initiative, Data Resource Center on Child and Adolescent Health Website. Available at http://www.nschdata.org/browse/survey/results?q=2390&r=1&g=451 Accessed 24 July 2015.

Negus, SS (2003) Rapid assessment of choice between cocaine and food in rhesus monkeys: Effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. Neuropsychopharmacology 28: 919-931.

Negus, SS, Mello, NK (2003a) Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys. Psychopharmacology (Berlin) 167: 324-333.

Negus, SS, Mello, NK (2003b) Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a second-order schedule in rhesus monkeys. Drug and Alcohol Dependence 70: 39-52.

Nelissen, K, Jarraya, B, Arsenault, JT, Rosen, BR, Wald, LL, Mandeville, JB, et al (2012) Neural correlates of the formation and retention of cocaine-induced stimulus-reward associations. Biological Psychiatry 72: 422-428.

Novick, A, Yaroslavsky, I, Tejani-Butt, S (2008) Strain differences in the expression of dopamine D1 receptors in Wistar-Kyoto (WKY) and Wistar rats. Life Sciences 83: 74-78.

Nussbaum, NL (2012) ADHD and female specific concerns: A review of the literature and clinical implications. Journal of Attention Disorders 16: 87-100.

Oakly, AC, Brox, BW, Schenk, S, Ellenbroek BA (2014) A genetic deletion of the serotonin transporter greatly enhances the reinforcing properties of MDMA in rats. Molecular Psychiatry 19: 534-535.

Ohno, Y, Okano, M, Masui, A, Imaki, J, Egawa, M, Yoshihara, C (2012) Region-specific elevation of D_1 receptor-mediated neurotransmission in the nucleus accumbens of SHR, a rat model of attention deficit/hyperactivity disorder. Neurpharmacology 63: 547-554.

Pamplona FA, Pandolfo P, Savoldi R, Prediger RD, Takahashi RN (2009) Environmental enrichment improves cognitive deficits in Spontaneously Hypertensive Rats (SHR): Relevance for Attention Deficit/Hyperactivity Disorder (ADHD). Progress in Neuropsychopharmacology and Biological Psychiatry 33: 1153-1160.

Pardon, M-C, Gould, GG, Garcia, A, Phillips, L, Cook, MC, Miller, A, et al (2002) Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: Implications for susceptibility to stress-related neuropsychiatric disorders. Neuroscience 115: 229-242.

Paré, W (1994) Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. Physiology and Behavior 55: 433-439.

Paré, W, Kluczynski, J (1997) Differences in the stress response of Wistar-Kyoto (WKY) rats from different vendors. Physiology and Behavior 62: 643-648.

Patrick, KS, Morowitz, JS (1997) Pharmacology of methylphenidate, amphetamine enantiomers and pemoline in Attention-Deficit Hyperactivity Disorder. Human Psychopharmacology 12: 527-546.

Pennick, M (2010) Absorption of lisdexamfetamine dimesylate and its enzymatic conversion to d-amphetamine. Neuropsychiatric Disease and Treatment 24: 317-327.

Piazza, PV, Deroche-Gamonent, V, Rouge-Pont, F, Le Moal, M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. Journal of Neuroscience 20: 4226-4232.

Pierce, RC, Born, B, Adams, M, Kalivas, PW (1996) Repeated intra-ventral tegmental area administration of SKF-38393 induces behavioral and neurochemical sensitization to a subsequent cocaine challenge. Journal of Pharmacology and Experimental Therapeutics 278: 384-392.

Pisanu, A, Lecca, D, Valentini, V, Bahi, A, Dreyer, JL, Cacciapaglia, F, et al (2015) Impairment of acquisition of intravenous cocaine self-administration by RNA-interference of dopamine D1-receptors in the nucleus accumbens shell. Neuropharmacology 89: 398-411.

Potvin, S, Stavro, K, Rizkallah, E, Pelletier, J (2014) Cocaine and cognition: A systematic quantitative review. Journal of Addiction Medicine 8: 368-376.

Ragozzino, ME, Detrick, S, Kesner, RP (1999) Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. Journal of Neuroscience 19: 4585-4594.

Raman, SR, Marshall, SW, Gaynes, BN, Haynes, K, Naftel, AJ, Stürmer, T (2015) An observational study of pharmacological treatment in primary care of children with ADHD in the United Kingdom. Psychiatry Services 66: 617-624

Ramos, M, Goñi-Allo, B, Aguirre, N (2005) Administration of SCH 23390 into the medial prefrontal cortex blocks the expression of MDMA-induced behavioral sensitization in rats: an effect mediated by 5-HT2C receptor stimulation and not by D1 receptor blockade. Neuropsychopharmacology 30: 2180-2191.

Ranaldi, R, Wise, RA (2001) Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. Journal of Neuroscience 21: 5841-5846.

Rapport, MD, Alderson, RM, Kofler, MJ, Sarver, DE, Bolden, J, Sims, V (2008) Working memory deficits in boys with attention-deficit/hyperactivity disorder (ADHD): The contribution of central executive and subsystem processes. Journal of Abnormal Child Psychology 36: 825-837.

Reeve, WV, Schandler, SL (2001) Frontal lobe functioning in adolescents with attention deficit hyperactivity disorder. Adolescence 36: 749-765.

Ricart, TM, De Niear, MA, Jiao, X, Pang, KC, Beck, KD, Servatius, RJ (2015) Deficient proactive interferene of eyeblink conditioning in Wistar-Kyoto rats. Behavioural Brain Research 216: 59-65.

Roberts, DC, Brebner, K, Vincler, M, Lynch, WJ (2002) Patterns of cocaine self-administration in rats produced by various access conditions under a discrete trials procedure. Drug and Alcohol Dependence 67: 291-299.

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

Robinson, TE, Berridge, KC (1993) The neural basis of drug craving: An incentive-sensitization theory of addiction. Brain Research Reviews 18: 247-291.

Roessner, V, Sagvolden, T, DasBanerjee, T, Middleton, FA, Faraone, SV, Walaas, SI, et al (2010) Methylphenidate normalizes elevated dopamine transporter densities in an animal model of the attention-deficit/hyperactivity disorder combined type, but not to the same extent in one of the attention-deficit/hyperactivity disorder inattentive type. Neuroscience 167: 1183-1191.

Routh, DK, Schroeder, CS (1976) Standardized playroom measures as indices of hyperactivity. Journal of Abnormal Child Psychology 4: 199-207.

Rubia, K (2011) "Cool" inferior frontostriatal dysfunction in attention-deficit/hyperactivity disorder versus "hot" ventromedial orbitofrontal-limbic dysfunction in conduct disorder: A review. Biological Psychiatry 69: e69-e87.

Rubia, K, Smith, AB, Brammer, MJ, Toone, B, Taylor, E (2005) Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. American Journal of Psychiatry 162: 1067-1075.

Rubia, K, Alegria, AA, Cubillo, AI, Smith, AB, Brammer, MJ, Radua, J (2014) Effects of stimulants on brain function in attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Biological Psychiatry 76: 616-628.

Russell, VA, Sagvolden, T, Borgå Johansen, E (2005) Animal models of attention-deficit hyperactivity disorder. Behavioral and Brain Functions 1: 9.

Sagvolden, T (2000) Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). Neuroscience and Biobehavioral Reviews 24: 31-39.

Sagvolden, T, Hendley, ED, Knardahl, S (1992) Behavior of hypertensive and hyperactive rat strains: hyperactivity is not unitarily determined. Physiology and Behavior 52: 49-57.

Sagvolden, T, Russell, VA, Aase, H, Borgå Johansen, E, Farshbaf, M (2005) Rodent models of Attention-Deficit/Hyperactivity Disorder. Biological Psychiatry 57: 1239-1247.

Sagvolden, T, Xu, T (2008) l-Amphetamine improves poor sustained attention while d-amphetamine reduces overactivity and impulsiveness as well as improves sustained attention in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). Behavioral and Brain Functions 4: 3.

Scahill, L, Carroll, D, Burke, K (2004) Methylphenidate: Mechanism of action and clinical update. Journal of Child and Adolescent Psychiatric Nursing 17: 85-86.

Schenk, S, Izenwasser, S (2002) Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. Pharmacology Biochemistry and Behavior 72: 651-657.

Scholl, JL, Renner, KJ, Forster, GL, Tejani-Butt, S (2010) Central monoamine levels differ between rat strains used in studies of depressive behavior. Brain Research 1355: 41-51.

Schroeder, SR, Milar, C, Wool, R, Routh, DK (1980) Multiple measurement, transsituational diagnosis, and the concept of generalized overactivity. Journal of Pediatric Psychology 5: 365-375.

Schwarz, A, Cohen, S (2013) ADHD seen in 11% of US children as diagnoses rise. New York Times. Available at http://www.nytimes.com/2013/04/01/health/more-diagnoses-of-hyperactivity-causing-concern.html Accessed 23 June 2015.

Seidman, LJ, Biederman, J, Faraone, SV, Weber, W, Ouelette, C (1997) Toward defining a neuropsychology of attention deficit-hyperactivity disorder: Performance of children and adolescents from a large clinically referred sample. Journal of Consulting and Clinical Psychology 65: 150-160.

Schilström, B, Yaka, R, Argilli, E, Suvarna, N, Schumann, J, Chen, BT, et al (2006) Cocaine enhances NMDA receptor-mediated currents in ventral tegmental area cells via dopamine D5 receptor-dependent redistribution of NMDA receptors. Journal of Neuroscience 26: 8549-8558.

Sibley, MH, Kuriyan, AB, Evans, SW, Waxmonsky, JG, Smith, BH (2014) Pharmacological and psychosocial treatments for adolescents with ADHD: An updated systematic review of the literature. Clinical Psychology Review 34: 218-232.

Simchon, Y, Weizman, A, Rehavi, M (2010) The effect of chronic methylphenidate administration on presynaptic dopaminergic parameters in a rat model for ADHD. European Neuropsychopharmacology 20: 714-720.

Sizoo, B, van den Brink, W, Koeter, M, Gorissen van Eenige, M, van Wijngaarden-Cremers, P, et al (2010) Treatment seeking adults with autism or ADHD and co-morbid Substance Use Disorder: Prevalence, risk factors and functional disability. Drug and Alcohol Dependence 107: 44-50.

Smith, A, Cubillo, A, Barrett, N, Giampietro, V, Simmons, A, Brammer, M, Rubia, K (2013) Neurofunctional effects of methylphenidate and atomoxetine in boys with

attention-deficit/hyperactivity disorder during time discrimination. Biological Psychiatry 74: 615-622.

Somkuwar, SS*, Jordan, CJ*, Kantak, KM, Dwoskin, LP (2013a) Adolescent atomoxetine treatment in a rodent model of ADHD: Effects on cocaine self-administration and dopamine transporters in frontostriatal regions. Neuropsychopharmacology 38: 2588-2597 *Co-first authors.

Somkuwar, SS, Darna, M, Kantak, KM, Dwoskin, LP (2013b) Adolescence methylphenidate treatment in a rodent model of attention deficit/hyperactivity disorder: Dopamine transporter function and cellular distribution in adulthood. Biochemical Pharmacology 86: 309-316.

Somkuwar, SS, Kantak, KM, Dwoskin, LP (2015) Effect of methylphenidate treatment during adolescence on norepinephrine transporter function in orbitofrontal cortex in a rat model of attention deficit hyperactivity disorder. Journal of Neuroscience Methods. Available 11 February 2015.

Spear, LP (2000) The adolescent brain and age-related behavioral manifestations. Neuroscience and Biobehavioral Review 24: 417-463.

Spencer, TJ, Brown, A, Seidman, LJ, Valera, EM, Makris, N, Lomedico, A, et al (2013) Effect of psychostimulants on brain structure and function in ADHD. Journal of Clinical Psychiatry 74: 902-917.

Stanger, C, Elton, A, Ryan, SR, James, GA, Budney, AJ, Kilts, CD (2013) Neuroeconomics and adolescent substance abuse: individual differences in neural networks and delay discounting. Journal of the American Academy of Child and Adolescent Psychiatry. 52: 747-755.

Stanis, JJ, Andersen, SL (2014) Reducing substance use during adolescence: a translational framework for prevention. Psychopharmacology (Berlin) 231: 1437-1453.

Steketee, JD, Kalivas, PW (2011) Drug wanting: Behavioral sensitization and relapse to drug-seeking behavior. Pharmacological Reviews 63: 348-365.

Steinhausen, H-C, Bisgaard, C (2014) Substance use disorders in association with attention-deficit/hyperactivity disorder, co-morbid mental disorders, and medication in a nationwide sample. European Neuropsychopharmacology 24: 232-241.

Substance Abuse and Mental Health Services Administration (SAMHSA; 2002) National and state estimates of the drug abuse treatment gap: 2000 National Household Survey on Drug Abuse. Office of Applied Studies, NSDUH Series H-14, DHHS Publication No. SMA 02-3640. Rockville, MD.

Substance Abuse and Mental Health Services Administration (SAMHSA; 2006) Results from the 2005 National Survey on Drug Use and Health: National findings. Office of Applied Studies, NSDUH Series H-30, DHHS Publication No. SMA 06- 4194. Rockville, MD.

Substance Abuse and Mental Health Services Administration (SAMHSA; 2013) Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings, Office of Applied Studies, NSDUH Series H-48, HHS Publication No. SMA 14-4863 Rockville, MD.

Sun H, Cocker PJ, Zeeb FD, Winstanley CA (2011) Chronic atomoxetine treatment during adolescence decreases impulsive choice, but not impulsive action, in adult rats and alters markers of synaptic plasticity in the orbitofrontal cortex. Psychopharmacology (Berlin) 219: 285-301.

Tegelbeckers, J, Bunzeck, N, Duzel, E, Bonath, B, Flechtner, HH, Krauel, K (2015) Altered salience processing in attention deficit hyperactivity disorder. Human Brain Mapping 36: 2049-2060.

Tejani-Butt, SM, Paré, WP, Yang, J (1994) Effect of repeated novel stressors on depressive behavior and brain norepinephrine receptor system in Sprague-Dawley and Wistar Kyoto (WKY) rats. Brain Research 649: 27-35.

Thanos, PK, Michaelides, M, Benveniste, H, Wang, GJ, Volkow, ND (2007) Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. Pharmacology Biochemistry and Behavior 87: 426-433.

Thomas, MJ, Beurrier, C, Bonci, A, Malenka, RC (2001) Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. Nature Neuroscience 4: 1217-1223.

Tomie, A, Grimes, KL, Poherecky, LA (2008) Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. Brain Research Reviews 58: 121-135.

Udvardi, PT, Föhr, KJ, Henes, C, Liebau, S, Dreyhaupt, J, Boeckers, TM, Ludolph, AG (2013) Atomoxetine affects transcription/translation of the NMDA receptor and the norepinephrine transporter in the rat brain--an in vivo study. Drug Design Development and Therapy 7: 1433-1446.

Unrod, M, Drobes, DJ, Stasieqicz, PR, Ditre, JW, Heckman, B, Miller, RR, et al (2013) Decline in cue-provoked craving during cue exposure therapy for smoking cessation. Nicotine Tobacco Research 16: 306-315.

Upadhyaya, HP, Rose, K, Wang, W, O'Rourke, K, Sullivan, B, Deas, D, Brady, KT (2005) Attention-Deficit/Hyperactivity Disorder, medication treatment, and substance use patterns among adolescents and young adults. Journal of Child and Adolescent Psychopharmacology 15: 799-809.

van den Bergh, FS, Bloemarts, E, Chan, JS, Groenink, L, Olivier, B, Oosting, RS (2006) Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. Pharmacology, Biochemistry, and Behavior 83: 380-390.

Vendruscolo, LF, Izidio, GS, Takahashi, RN, Ramos, A (2008) Chronic methylphenidate treatment during adolescence increases anxiety-related behaviors and ethanol drinking in adult spontaneously hypertensive rats. Behavioral Pharmacology 19: 21-27.

Vendruscolo, LF, Izidio, GS, Takahashi, RN (2009) Drug reinforcement in a rat model of attention deficit/hyperactivity disoder - the Spontaneously Hypertensive Rat (SHR). Current Drug Abuse Reviews 2: 177-183.

Vezina, P (1996) D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. Journal of Neuroscience 16: 2411-2420.

Visser, SN, Danielson, ML, Bitsko, RH, Holbrook, JR, Kogan, MD, Ghandour, RM, et al (2014) Trends in the parent-report of health care provider-diagnosed and medicated attention-deficit/hyperactivity disorder: United States, 2003-2011. Journal of the American Academy of Child and Adolescent Psychiatry 53:34-46e2.

Volkow, ND, Swanson, JM (2008) Does childhood treatment of ADHD with stimulant medication affect substance abuse in adulthood? American Journal of Psychiatry 165: 553-555.

Volkow, ND, Ding, YS, Fowler, JS, Wang, GJ, Logan, J, Gatley, JS, et al. (1995) Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Archives of General Psychiatry 52: 456-463.

Volkow, ND, Wang, G-J, Newcorn, JH, Kollins, SH, Wigal, TL, Telang, F, et al (2010) Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. Molecular Psychiatry 16: 1146-1154.

Vonmoos, M, Hulka, LM, Preller, KH, Jenni, D, Schulz, C, Baumgartner, MR, Quednow, BB (2013) Differences in self-reported and behavioral measures of impulsivity in recreational and dependent cocaine users. Drug and Alcohol Dependence 133: 61-70.

von Rhein, D, Cools, R, Zwiers, MP, van der Schaaf, M, Franke, B, Luman, M (2015) Increased neural responses to reward in adolescents and young adults with attention-deficit/hyperactivity disorder and their unaffected siblings. Journal of the American Academy of Child and Adolescent Psychiatry 54: 394-402.

Warton, FL, Howells, FM, Russell, VA (2009) Increased glutamate-stimulated release of dopamine in substantia nigra of a rat model for attention-deficit/hyperactivity disorder—lack of effect of methylphenidate. Metabolic Brain Disease 24: 599-613.

Watanabe, Y, Fujita, M, Ito, Y, Okada, T, Kusuoka, H, Nishimura, T (1997) Brain dopamine transporter in spontaneously hypertensive rats. Journal of Nuclear Medicine 38: 470-474.

Wickens, JR, Hyland, BI, Tripp, G (2011) Animal models to guide clinical drug development in ADHD: Lost in translation? British Journal of Pharmacology 164: 1107-1128.

Wilcox, KM, Lindsey, KP, Votaw, JR, Goodman, MM, Martarello, L, Carroll, FI, Howell, LL (2002) Self-administration of cocaine and the cocaine analog RTI-113: relationship to dopamine transporter occupancy determined by PET neuroimaging in rhesus monkeys. Synapse 43: 78-85.

Wilcox, CE, Teshiba, TM, Merideth, F, Ling, J, Mayer, AR (2011) Enhanced cue reactivity and fronto-striatal functional connectivity in cocaine use disorders .Drug and Alcohol Dependence 115: 137-144.

Wilens, T (2006) Mechanism of action of agents used in Attention-Deficit/Hyperactivity Disorder. Journal of Clinical Psychiatry 67: 32-37.

Wilens, TE, Faraone, SV, Biederman, J, Gunawardene, S (2003) Does stimulant therapy of Attention-Deficit/Hyperactivity Disorder beget later substance abuse? A meta-analytic review of the literature. Pediatrics 111: 179-185.

Wilens, TE, Hammerness, P, Utzinger, L, Schillinger, M, Georgiopoulous, A, Doyle, RL, et al (2009) An open study of adjunct OROS-methylphenidate in children and adolescents who are atomoxetine partial responders: I effectiveness. Journal of Child and Adolescent Psychopharmacology 19: 485-492.

Wilens, TE, Martelon, MK, Joshi, G, Bateman, C, Fried, R, Petty, C, Biederman, J (2011) Does ADHD predict substance-use disorders? A 10-year follow-up study of young adults with ADHD. Journal of the American Academy of Child Adolescent Psychiatry 50: 543-553.

Wooters, TE, Bardo, MT (2011) Methylphenidate and fluphenazine, but not amphetamine, differentially affect impulsive choice in Spontaneously Hypertensive, Wistar-Kyoto and Sprague-Dawley rats. Brain Research 1396: 45-53.

Wyss, JM, Kadish, I, van Groen, T (2003) Age-related decline in spatial learning and memory: attenuation by captopril. Clinical and Experimental Hypertension 25: 455-474.

Xie, Z, Miller, GM (2009) A receptor mechanism for methamphetamine action in dopamine transporter regulation in brain. Journal of Pharmacology and Experimental Therapeutics 330: 316-325.

Xie, Z, Westmoreland, SV, Bahn, ME, Chen, GL, Yang, H, Vallender, EJ, et al (2007) Rhesus monkey trace amine-associated receptor 1 signaling: Enhancement by monoamine transporters and attenuation by the D2 autoreceptor in vitro. Journal of Pharmacology and Experimental Therapeutics 321: 116-127.

Yager, LM, Robinson, TE (2013) A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. Psychopharmacology 226: 217-228.

Zahniser, NR, Sorkin, A (2004) Rapid regulation of the dopamine transporter: Role in stimulant addiction? Neuropharmacology 47: 80-91.

Zetterqvist, J, Asherson, P, Halldner, L, Långström, N, Larsson, H (2012) Stimulant and non-stimulant attention deficit/hyperactivity disorder drug use: Total population study of trends and discontinuation patterns 2006-2009. Acta Psychiatrica Scandinavica 128: 70-77.

Zhang-James, Y, Yang, L, Middleton, FA, Yang, L, Patak, J, Faraone, SV (2014) Autism-related behavioral phenotypes in an inbred rat substrain. Behavioural Brain Research 269: 103-114.

Zimmer, BA, Chiodo, KA, Roberts, DC (2014) Reduction of the reinforcing effectiveness of cocaine by continuous D-amphetamine treatment in rats: importance of active self-administration during treatment period. Psychopharmacology (Berlin) 231: 949-954.

CURRICULUM VITAE