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Author
Robertson, Chelsea Lyn

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Striatal Dopamine Receptors, Inhibitory Control
and Methamphetamine Use Disorder

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Molecular and Medical Pharmacology

by

Chelsea Robertson

2015
ABSTRACT OF THE DISSERTATION

Striatal Dopamine Receptors, Inhibitory Control
and Methamphetamine Use Disorder

by

Chelsea Robertson
Doctor of Philosophy in Molecular and Medical Pharmacology
University of California, Los Angeles, 2015
Professor Edythe D. London, Chair

Inhibitory control is a neurocognitive construct that describes the capacity to exert control over behaviors, thoughts, actions and emotions. It is essential to everyday life and is an important component of executive function necessary for goal directed behavior. However, deficits in inhibitory control are manifested across several neuropsychiatric disorders, especially substance use disorders. Although the neurobiological mechanisms that underlie human inhibitory control are not completely clear, neuroimaging studies have advanced our understanding of the underlying circuitry of inhibitory control and pharmacological studies have highlighted an essential role for dopaminergic neurotransmission. However, there remains much to be understood about how different striatal dopaminergic receptor systems influence inhibitory control capacity. Therefore, the research in this dissertation aims to increase our understanding of how striatal
dopaminergic signaling at different dopamine receptor subtypes (D1- and D2-type) contributes to individual levels of impulsivity and inhibitory control.

Dopaminergic deficiencies in stimulant use disorder are linked to several clinically relevant indices, including treatment retention and outcomes. Dopamine markers are associated with behaviors and cognitive functions important to the development and maintenance of addiction, including inhibitory control, risk-taking and delay of gratification. Therefore, augmenting dopaminergic function is an attractive therapeutic target in the treatment of stimulant use disorders. However, the lack of approved pharmacological agents for this purpose has spurred an interest in alternative approaches to augmenting the dopaminergic signaling in stimulant users. On the basis of a large animal literature, aerobic exercise has been suggested as a mechanism to increase dopamine system function in stimulant use disorder. Research in this dissertation aims to determine the effects of exercise on the dopamine system in individuals with methamphetamine use disorder.

Determining the relevance of individual variation in dopaminergic markers on human behavior will improve our understanding of the molecular basis of inhibitory control and its dysregulation in substance use disorders. Furthermore, investigating methods to improve dopamine function in individuals with methamphetamine use disorder represents an important step for improving addiction treatment.
This dissertation of Chelsea Robertson is approved.

James David Jentsch

Daniel H. Silverman

Richard W. Olsen

Heather R. Christofk

Edythe D. London, Committee Chair

University of California, Los Angeles

2015
To my family and friends…

Your endless love, support and encouragement were integral to this work.

Without it, this dissertation would not have been possible.

Thank you.
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Some chapters of this dissertation are versions of published, or submitted manuscripts.


Curriculum Vitae

Chelsea Robertson

Education
UC Los Angeles, PhD candidate, Molecular and Medical Pharmacology, 2015
UC Santa Barbara, BSc in Pharmacology; Minor in Exercise and Health Science, 2006

Recent Research Experience
Graduate Student Researcher, UCLA Laboratory of Molecular Neuroimaging, 2010 – 2015
Under the mentorship of Dr. Edythe London, utilized multimodal neuroimaging approaches to investigate the neurocircuitry underlying self-control and impulsivity. Examined the link between dopamine system markers and neurocognitive function that are important in addictive behaviors.

Senior Research Associate, Gilead Sciences, Palo Alto, CA 2007 – 2009
Examining the in-vivo effects of novel compounds (cardiac sodium channel blockers) on ischemia and reperfusion injury in rodent models. Assessing the efficacy of investigational compounds on Beta-islet insulin secretion and glucose tolerance in diabetic rat and obese mouse models.

Teaching Experience
Teaching Assistant, Functional Neuroanatomy, UCLA Fall, 2013
Teaching Assistant, Pharmacology Laboratory, UCSB Fall & Winter 2007

Lectures, Grants and Awards
• Los Angeles County Unified School District Educational Outreach Lecture, 2011. “Cigarette Smoking and the Adolescent Brain”
• UCLA Molecular and Medical Pharmacology Department Travel Award, 2011.
• NeuroReceptor Mapping of the Living Brain (NRM) Young Investigators Award, 2012.
• College of Problems on Drug Dependence “Late-Breaking” Research Selection, 2012.
• UCLA Brain Research Institute Neuroscience Graduate Student Travel Award, 2013.

Original Research Articles


**Book Chapter**


**Selected Conference Abstracts**


CHAPTER 1: INTRODUCTION
METHAMPHETAMINE USE: A GLOBAL HEALTH PROBLEM

Amphetamine-type stimulants, including methamphetamine, are among the most commonly abused illicit drugs worldwide, second only to cannabis (UNDOC, 2014). The World Health Organization estimates the number of amphetamine users to be more than 35 million globally, more than that of cocaine (15 million) and heroin (10 million) combined (UNODC, 2012). In 2013, global seizures of amphetamine-type stimulants reached an all-time high of 144 tons, up 15 per cent from 2011, due in large part to increases in methamphetamine seizures (UNDOC, 2014).

Methamphetamine use is widespread in the United States, with the highest prevalence of use in the western states and the Midwest (SAMHSA, 2014a). In 2013, 1.4 million Americans reported using methamphetamine in the last year before participating in a survey and 595,000 people who reported using methamphetamine in the past month (595,000)(SAMHSA, 2014a). In 2012, approximately 6.1% of admissions to publicly funded substance treatment facilities in the United States were for methamphetamine use, an increase from 2002 (SAMHSA, 2014b). In California, treatment center admission rates for methamphetamine use was higher than for that of any other drug from 2002-2013 (SAMHSA, 2014b).

Methamphetamine abuse is a substantial public health problem (Gonzales et al., 2010). The estimated economic cost of methamphetamine use was estimated at $23 billion in 2005, largely due to pre-mature mortality, crime and criminal justice costs (Nicosia et al., 2009) and physical health hazards related to methamphetamine production (Vearrier et al., 2012).
PHARMACOLOGY OF METHAMPHETAMINE

Mechanism of action

Similar to other drugs of abuse, methamphetamine administration increases synaptic dopamine concentrations in the ventral striatum (Di Chiara and Imperato, 1988). Methamphetamine acts as an indirect agonist at dopaminergic, serotonergic and noradrenergic terminals. The structural similarity to endogenous monoamines (Figure 1) allows methamphetamine to substitute for dopamine, serotonin or norepinephrine at pre-synaptic terminal transporters and at vesicular monoamine transporters (VMAT) (Cruickshank and Dyer, 2009). Once inside the pre-synaptic vesicle, the weak-base properties of methamphetamine disrupt the pH gradient that sequesters monoamines inside the vesicular membrane. Monoamines are then displaced from the neurotransmitter vesicle and released into the cytosol, increasing neurotransmitter concentrations inside the presynaptic terminal (Kish, 2008). Rising intracellular neurotransmitter concentrations reverse the endogenous action of presynaptic transporters of dopamine, serotonin or norepinephrine (DAT, SERT, NET), and monoamines are reverse-transported from the cytosol through the terminal transporters into the synapse (Kish, 2008, Cruickshank and Dyer, 2009). Methamphetamine also inhibits monoamine oxidase, the major metabolic enzyme for monoamines in the synapse (Kish, 2008, Cruickshank and Dyer, 2009). The relative affinities for methamphetamine of the different monoamine transporters in brain make the effects of the drug on norepinephrine twice the effect on dopamine, and sixty times the effect on serotonin (Cruickshank and Dyer, 2009). However, the effects on dopamine appear to be the most neurotoxic.
The neurotoxic effects of methamphetamine exposure have been studied in animal models. Exposure to methamphetamine leads to damage selective to the dopaminergic terminals, sparing the cell bodies. The damage to dopaminergic terminals is due to dopamine auto-oxidation in the cytosol and synapse. Oxidation of dopamine or its metabolites generates reactive oxygen species, including 6-hydroxydopamine and H$_2$O$_2$, causing oxidation of cytosolic proteins and lipids (Davidson et al., 2001). Furthermore, methamphetamine administration can also disrupt mitochondrial function in monoaminergic cells by dissipating the mitochondrial electron gradient established by the electron transport chain, eventually leading to apoptosis.

Administration of high doses of methamphetamine increase core body temperature and cause hyperthermia, which contributes to the molecular mechanisms by which methamphetamine neurotoxicity occurs, including increased oxidative stress and mitochondrial dysfunction (Yamamoto et al., 2010). For example in mice, high-dose methamphetamine injections administered at room temperature produced a significant depletion of dopamine in the striatum. However, when the same treatment was administered in a cold environment, striatal dopamine
depletions was attenuated (Ali et al., 1994). Recent literature has identified several mechanisms of methamphetamine neurotoxicity that likely reflect a combination of excitotoxic, proteolytic, inflammatory, and bioenergetic processes (Yamamoto et al., 2010, Kelly, 2011, Yu et al., 2015). While not directly testable in human subjects, molecular imaging data from human subjects shows that chronic methamphetamine use is associated with markers of neuroinflammation ([11C]PK11195 binding) (Sekine et al., 2008) and metabolic abnormalities (cerebral glucose metabolism measured with [18F]-deoxyglucose) (London et al., 2004, London et al., 2005).

**Pharmacokinetics**

The most commonly used routes of administration of methamphetamine are oral, intranasal, intravenous and by smoking (Kelly, 2011). The pharmacokinetics of methamphetamine varies across routes of administration. Maximum plasma concentrations are reached most rapidly after intravenous administration (6 minutes) and most slowly after oral ingestion (~215 minutes) (Cruickshank and Dyer, 2009). The plasma elimination half-life of methamphetamine is between 9-12 hours in human subjects depending on route of administration (Kish, 2008, Cruickshank and Dyer, 2009).

Peak subjective effects occur within 15 minutes of administration and dissipate after about 4-5 hours. The large dissociation between the peak plasma concentrations and peak subjective effects indicates a certain degree of acute tolerance resulting from rapid molecular adaptations, such as down-regulation of monoamine release or receptor internalization (Volkow et al., 2011). Notably, the cardiovascular effects of methamphetamine tend to persist after the subjective effects have faded. Diminishing subjective effects may promote repeated dosing every
4-5 hours, while the cardiovascular risks may continue to increase (Cruickshank and Dyer, 2009).

**Behavioral and physiological effects in humans**

Doses of methamphetamine studied in the clinical laboratory are sizably lower than reported street doses. Doses administered to research participants range from 10 mg-50 mg, in comparison self-reported doses ranging from 50-500 mg per use, with repeated dosing totaling up to 4 grams per day (Cruickshank and Dyer, 2009, Carvalho et al., 2012). Moderate doses of methamphetamine used in research settings (< 30mg) produce increased heart rate, blood pressure and body temperature, as well as pupillary dilation and reduced appetite (Carvalho et al., 2012). Behavioral effects include heightened arousal, euphoria, positive mood and reduced fatigue and appetite, with increased anxiety reported as the main negative effect. Larger doses (< 50-600 mg) produce initial hypertension and increased positive effects, followed by psychotic symptoms, including paranoia, as well as aggressive behaviors, violent thoughts and excessive disordered speech (Carvalho et al., 2012).

Withdrawal from methamphetamine produces a distinct physiological and cognitive profile (Carvalho et al., 2012). Symptoms include sleep disturbances, reduced energy, depressed mood, heightened anxiety, hyperphagia, agitation, vivid or unpleasant dreams and suicidal ideation. The severity of the methamphetamine withdrawal syndrome often is related to the duration and frequency of use (Carvalho et al., 2012).
METHAMPHETAMINE USE DISORDER

Clinical Features

Long-term use of methamphetamines is associated with a number of physiological and cardiovascular problems, including hypertension, chronic heart failure, changes in cognitive function and personality disorders (Carvalho et al., 2012). Abrupt cessation of chronic methamphetamine use can produce psychotic symptoms. Auditory, visual and tactile hallucinations, delusions, and odd speech may persist for up to 4 weeks of abstinence. Chronic exposure also increases cardiovascular complications, including chronic hypertension, coronary artery disease, cardiomyopathy, stroke or cardiac sudden death (Cruickshank and Dyer, 2009).

Depressed mood and heightened anxiety follow the cessation of chronic methamphetamine use, with severe symptoms occurring at 2-3 days of abstinence and resolving within about two weeks (Zorick et al., 2010). Moderate levels of neuropsychological impairment are also associated with methamphetamine use (Scott et al., 2007, Simon et al., 2010, Dean et al., 2013). Impairments in impulse control, memory recall, and sustained attention and working memory are linked to chronic methamphetamine use (London et al., 2004, London et al., 2005). These findings correspond with clinical observations that methamphetamine-dependent patients tend to present as distractible and have difficulty sustaining attention (Cruickshank and Dyer, 2009).

Neurobiological features

Chronic exposure to cocaine or methamphetamine and repeated perturbation of the dopaminergic system produce sequential neurochemical adaptations that down-regulate
dopaminergic function and persist long after the cessation of drug use (Volkow et al., 2011). Neurochemical changes occur in the circuits that mediate reward/saliency, motivation/drive, inhibitory control/executive functions, all of which are modulated by dopaminergic pathways (Koob and Volkow, 2010).

Molecular abnormalities within the dopaminergic system in stimulant addicts are widely studied using molecular imaging techniques (Bonci, 2013). Human PET studies show alterations to several markers of the dopaminergic function in stimulant users when compared to non-drug-using control subjects, and a consistent finding across studies is evidence for low striatal D2-type dopamine receptors (Broft and Martinez, 2012, Volkow et al., 2015). Chronic users of cocaine (Volkow et al., 1990) and methamphetamine (Lee et al., 2009) show deficits in striatal D2-type receptor availability, however poly-substance users show higher binding of a D3-type receptor preferring ligand. ([11C-PHNO]) (Boileau et al., 2012), when compared to control subjects. In addition, chronic stimulant users show abnormalities in striatal levels of vesicular monoamine transporters (VMAT) (Johanson et al., 2006, Boileau et al., 2008, Narendran et al., 2012) and dopamine transporters (DAT) (McCann et al., 1998, Sekine et al., 2001, Volkow et al., 2001a). PET studies have also suggested that stimulant use disorders are associated with diminished activity of the dopaminergic presynaptic element (Volkow et al., 1997, Wang et al., 2012, Volkow et al., 2014), low levels of dopamine synthesis (Baxter et al., 1988) and diminished endogenous striatal dopamine levels (Martinez et al., 2009).

Stimulant addiction (methamphetamine or cocaine) is also associated with neurotransmitter abnormalities outside of the dopamine system. Deficits in serotonin transporter binding (SERT), assessed with PET and [11C]-McN5652, are seen in subcortical brain regions of
methamphetamine users (Sekine et al., 2006). Cocaine users show higher levels of mu-receptor binding than controls, when measured using $[^{11}\text{C}]$-carfentanil, in the cingulate, frontal cortex, caudate and thalamus, and this effect persists for up to 12 weeks of abstinence (Zubieta et al., 1996, Gorelick et al., 2005). Endogenous opiate-receptor signaling represents an important facet of substance dependence because many studies show relationships between mu-receptor levels and drug craving (Zubieta et al., 1996, Gorelick et al., 2005) and substance abuse treatment outcomes (Ghitza et al., 2010). Although several neurotransmitter systems are affected in addiction, dopamine shows the most widespread effects.

The commonality of dopaminergic deficits across several addictive disorders raises questions regarding the extent to which these differences pre-date drug exposure in human subjects (Volkow et al., 2015). Although not directly testable in humans, mounting evidence from animal studies suggest that the hypodopaminergic function observed in human addicts is likely partially due to the effects of chronic drug exposure. Studies investigating effects of chronic methamphetamine exposure show lasting changes to neurochemistry and brain structure (Groman et al., 2012, Groman et al., 2013). Vervet monkeys exposed to a methamphetamine-dosing regimen designed to mimic human drug consumption patterns showed significant drug-induced decreases in striatal D2-type receptors that persisted for at least seven weeks after cessation of treatment (Groman et al., 2012). In another study, chronic exposure to cocaine significantly reduced D2-type receptor availability, and these reductions remained detectable for 7 months post-exposure (Nader and Czoty, 2005). Taken together with findings from human studies, these data suggest that the dopaminergic deficits observed in human addicts are, in part, due to chronic drug use.
However, potentially pre-existing individual differences in markers of the striatal dopaminergic system, such as low D2-type receptor levels, are of particular interest in understanding the vulnerability to develop substance use disorders (Volkow et al., 2015). Identification of low dopamine signaling as a vulnerability factor to substance dependence is supported by observations from a study of non-alcoholic subjects with a strong family history of alcoholism (high-risk individuals) (Volkow et al., 2006). Despite an association of alcoholism with low striatal D2-type receptors, high-risk non-alcoholics have higher striatal D2-type receptors than their alcoholic family members, and higher D2-type receptors than non-alcoholics from low-risk families (Volkow et al., 2006), suggesting that this neurochemical phenotype may protect against alcohol dependence. These findings are supported by animal studies showing that animals with low levels of striatal D2-type receptors are more likely to self-administer stimulants (Dalley et al., 2007). Furthermore, rats exhibit decreased alcohol self-administration after undergoing an increase in striatal D2 receptor expression (Thanos et al., 2001).

Additional evidence of a protective effect of high striatal dopamine signaling against drug use comes from PET studies showing that individuals with high D2-type receptor availability report more unpleasant subjective drug effects, suggesting that they may be less vulnerable to stimulant abuse (Volkow et al., 1999, Volkow et al., 2002b). For example, when methylphenidate, a stimulant with a mechanism of action similar to that of cocaine, was administered to healthy control subjects, an inverse relationship was found between D2-type receptor availability and subjective drug experience. Individuals with high D2-type receptor availability reported more unpleasant effects, suggesting a lower risk of drug self-administration (Volkow et al., 2002b). This idea was supported by work using animal models (Nader and Czoty, 2005, Cumming et al., 2011, Gould et al., 2012), showing that striatal D2-type receptor
availability, quantified in drug-naïve rhesus monkeys with PET and $[^{18}\text{F}]-\text{FCP}$, was negatively correlated with cocaine self-administration.

Animal studies of social hierarchies have provided insight into other factors, particularly environmental influences that contribute to drug abuse vulnerability and D2-type receptor availability. Social dominance and subordinance in animals are considered forms of environmental enrichment and stress, respectively. Dominant social status is associated with higher $[^{11}\text{C}]-\text{raclopride}$ binding than subordinate social status in animals (Morgan et al., 2002, Czoty et al., 2010, Nader et al., 2012). The development of social dominance socially-housed cages increased dopamine D2-type receptor availability by approximately 20 percent, with no change in the subordinates (Morgan et al., 2002). Dominant male monkeys subsequently self-administered less cocaine than subordinates, suggesting protection against the reinforcing properties of cocaine induced by environmental changes (Morgan et al., 2002). Although it is unknown if changes in D2-type receptors in humans can be modulated by changes in the environment, studies in human subjects show positive correlations between D2-type receptor availability and measures of social support and social status (Martinez et al., 2010) and harm-avoidance (Kim et al., 2011). Taken together, these studies suggest dopamine receptors may provide a molecular marker that reflects an interaction between environment and genetic predisposition towards drug abuse (Martinez et al., 2010).

*Treatment options for methamphetamine dependence*

The standard of care for drug addictions is cognitive behavioral therapy. While certain types of treatment models show greater efficacy than others (Rawson et al., 2004, Rawson et al., 2006), increasing time spent in treatment and combining behavioral approaches has been show to
improve treatment outcomes (Rawson et al., 2006, Shoptaw et al., 2008, Brecht and Herbeck, 2014). However, behavioral approaches remain only moderately effective, as relapse rates to methamphetamine use were approximately 61% at a 1-year post-treatment discharge, and 14% at 3 years post-follow up (Brecht and Herbeck, 2014). There are no approved pharmacotherapies for the treatment of stimulant dependence (Karila et al., 2010, Forray and Sofuoglu, 2014) and despite substantial clinical efforts drugs targeting the dopamine system have shown little success in improving behavioral treatment outcomes for stimulant dependence (Rawson et al., 2002, Ling et al., 2006, Heinzerling et al., 2010). Therefore the need to identify techniques to bolster retention and engagement is critical to improve the success rates of currently available treatment.

The highly heterogenous nature of addictive disorders presents challenges for clinical trials (Karila et al., 2010). Hence, identifying the individual differences in molecular or cognitive profiles associated with response to treatment represents an important step towards improving treatment efficacy (Jupp and Dalley, 2014, Volkow et al., 2015). Selecting treatment combinations according to individual genetic make-up or molecular or neurocognitive phenotypes can advance addiction therapy and increase treatment efficacy. By extension, identifying pre-existing predictive risk factors for substance use later in life would bolster the development of better prognostic targets (Jupp and Dalley, 2014, Volkow et al., 2015).

Correlates of therapeutic success

Some cognitive and biological factors are predictive of retention in treatment and successful treatment outcomes. Dopaminergic deficits are a core clinical feature of stimulant use disorders (Volkow et al., 2009, Bonci, 2013) and these deficits show some clinically relevance. D2-type receptor availability and even more so, function of the striatal dopaminergic presynaptic
element, as inferred from dopamine release, is positively associated with retention and treatment outcomes for stimulant users (Martinez et al., 2011, Wang et al., 2012), suggesting that improving dopamine signaling would increase retention in treatment and improve outcomes. Therefore, increasing striatal dopaminergic signaling represents a potentially important therapeutic target in the care of individuals with addiction (Wang et al., 2004).

Methamphetamine dependence is associated with cognitive deficits in several domains, including learning, memory, executive functioning and processing speed (Scott et al., 2007, Simon et al., 2010, Dean et al., 2013). While some individuals show improvements in cognition with prolonged methamphetamine-abstinence (1 year), poor cognitive function is linked to poor treatment outcomes for stimulant dependence (Dean et al., 2009, Sofuoglu, 2010). Upon entry to a treatment program for cocaine dependence, individuals with lower scores on tests of executive function, memory and processing speed were less likely to complete the treatment program when compared to those with higher scores (Aharonovich et al., 2003). Similarly, self-reported impulsivity (Moeller et al., 2001, Patkar et al., 2004, Winhusen et al., 2013) and inhibitory control (Aharonovich et al., 2006, Turner et al., 2009, Stevens et al., 2014) are also linked to retention in treatment for substance use disorders. These findings imply that improving cognitive and executive function during early abstinence may improve retention and participation in behavioral treatment programs. Therefore understanding the biological commonality between impulsivity and stimulant abuse remains an important question in the pursuit of increasing the efficacy of current treatment approaches for substance addiction.
Inhibitory control is a neurocognitive construct that describes the capacity to exert control over behaviors, thoughts, actions and emotions (Dalley et al., 2008). Inhibitory control, or self-control, is a part of daily functioning and is an important component of goal-directed behavior. In contrast, impulsivity is defined as a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard for the negative consequences of these reactions to the individual or others (Moeller et al., 2001). Inhibition and self-control are essential to everyday life and play an important role in goal-directed behavior (Evenden, 1999, Bari and Robbins, 2013, Jentsch et al., 2014). There is a large degree of variation in impulsivity among the general population, however, excessive disinhibition and heightened impulsiveness are hallmark clinical features of substance use disorders (Bari and Robbins, 2013).

Inhibitory control in methamphetamine use disorder

When compared to control subjects, individuals with stimulant use disorders consistently show impairments in performance across domains of inhibition (Fillmore and Rush, 2002, Ersche et al., 2011, Weafer et al., 2014). Specifically, individuals with methamphetamine use disorder show poor motor response inhibition (London et al., 2005, Monterosso et al., 2005), steep discounting of delayed rewards (Monterosso et al., 2007), poor cognitive flexibility (Ghahremani et al., 2010), impaired emotion regulation (Tabibnia et al., 2011), and heightened self-reported impulsiveness and craving (Semple et al., 2005, Lee et al., 2009, Morales et al., 2015). The link between stimulant dependence and impulsivity implies a certain degree of intersection in the neurocircuitry involved in both processes, and that the neurobiology
subserving impulsivity is also involved in the etiology of drug addiction (Bari and Robbins, 2013, Jentsch et al., 2014).

Data from human cross-sectional studies show group differences between stimulant-dependent and healthy cohorts, but these findings cannot distinguish if the differences were a consequence of chronic drug use, or alternatively, were pre-existing to the initiation of drug use. Data from prospective and longitudinal studies suggest that pre-existing individual variation in inhibitory control in adolescents can predict subsequent future drug use (Kirisci et al., 2007, Fernie et al., 2013). Animal studies indicate that chronic stimulant exposure increases impulsiveness and reduces inhibition (Groman et al., 2012, Groman et al., 2013), and that pre-existing impulsivity is associated with enhanced drug self-administration (Dalley et al., 2008). In combination with the existing literature, these findings converge upon the understanding that heightened impulsivity is a both a cause and consequence of drug abuse (Jentsch and Taylor, 1999, de Wit, 2009).

*Types of inhibition*

Inhibitory control is a multidimensional behavioral construct and several different forms of self-control have been described in the literature (Evenden, 1999). While there are numerous different taxonomies that stratify impulsiveness, there are a few dominant varieties discussed in the literature. Impulsive behavior can be classified as behavioral, which involves control over actions and impulses; affective, which is control over emotions and feeling-states; and cognitive, which reflects decision-making, response flexibility, and control over thought processes (Gullo and Potenza, 2014, Jupp and Dalley, 2014). Not all types of impulsivity infringe on everyday
functions. Some aspects, such as “functional” impulsivity (Dickman, 1990) describe the proclivity to take advantage of fleeting or temporary favorable opportunities.

Neurocognitive tests and personality assessments are used to measure aspects of impulsivity and inhibition-related behaviors and neurocognitive constructs (Weafer et al., 2014). Motor inhibitory control is assessed by tests designed to measure the ability to withhold or cancel a planned motor response (Stop-signal Task; (Aron, 2007, Verbruggen and Logan, 2008); Continuous performance test (Homack and Riccio, 2006)). Affective inhibitory control is measured as the ability to suppress a default emotional response to unpleasant stimuli (Emotion Regulation task (Kim and Hamann, 2007)). Cognitive flexibility is assessed by tests that measure perseverative responding to a previously rewarded stimulus, when stimulus association rules have changed (Reversal Learning task (Cools et al., 2002)). Deferred gratification and temporal discounting of rewards, are other related forms of self-control that are typically indexed by measuring the degree to which an individual prefers smaller immediate rewards to rewards available after a delay (Delay Discounting task or monetary-choice questionnaire (Kirby et al., 1999). Finally, relevant personality scales measure the degree to which an individual endorses statements regarding impulsive behavior, for example “I make decisions quickly,” “I often buy things I cannot afford,” or “I act without thinking” (Patton et al., 1995, Evenden, 1999).

Several lines of evidence suggest that different kinds of inhibitory control share underlying neural processes and recruit common neural substrates (Kim and Hamann, 2007, Cohen and Lieberman, 2010, Tabibnia et al., 2011). Some studies report relationships between outcome variables from different assessments of impulsivity (Reynolds et al., 2006, Weafer et al., 2013). However the recent use of non-invasive neuroimaging has helped to identify brain
regions that are involved in inhibitory control. Frontostriatal circuitry was identified as contributing to action inhibition (Aron, 2007, Ghahremani et al., 2012), affective inhibition (Tabibnia et al., 2011), cognitive flexibility (Ghahremani et al., 2010, Ersche et al., 2013) and delay discounting (Monterosso et al., 2007). These studies identify frontostriatal circuitry – and dopaminergic transmission in particular – as common neural substrates that underlie inhibitory control function (Groman and Jentsch, 2013, Volkow et al., 2015).

Stimulant dependence is often linked with structural, functional and molecular abnormalities within frontostriatal circuitry (Chang et al., 2007, Koob and Volkow, 2010). Neuroimaging studies report alterations in corticostratal glucose metabolism (Volkow et al., 2001b, London et al., 2005) and deficits in frontal grey matter (Thompson et al., 2004, Morales et al., 2012). Moreover, striatal dopaminergic hypofunction is consistently reported in stimulant dependence (Volkow and Baler, 2014) (Volkow, 2014), with striatal dopaminergic deficits linked to orbitofrontal dysfunction in stimulant use disorders (Volkow et al., 1993, Volkow et al., 2001b) on the basis of a correlation between striatal D2-type receptor availability and glucose metabolism in the orbital frontal cortex. These data suggest a functional relationship between striatal dopamine signaling and frontocortical function. Functional and molecular aberrations within the frontostriatal circuitry may underlie poor inhibition and elevated impulsivity in those diagnosed with stimulant-use disorders (Jentsch and Taylor, 1999, Fillmore and Rush, 2002, Ersche et al., 2011, Weafer et al., 2014)

*Striatal dopaminergic signaling*

Striatal dopaminergic signaling is an important modulator of the frontostriatal system and it plays a critical role in impulsive behavior and inhibitory control (Jentsch and Taylor, 1999,
Jentsch et al., 2014). In the striatum, dense dopaminergic innervation from midbrain dopamine neurons modulates activity in the direct and indirect striatal output pathways (Gerfen and Surmeier, 2011). Non-overlapping expression of dopamine receptor subtypes is the basis by which dopamine differentially regulates activity in the direct and indirect pathways (Gerfen, 2000, Smith and Kieval, 2000). D1-type receptors are selectively expressed on neurons in the direct pathway, and activation of D1 receptors increases their excitability. In contrast, D2 receptor activation decreases the excitability of neurons in the indirect pathway (Gerfen, 2000, Gerfen and Surmeier, 2011). The balance between striatal output pathways has been shown to be important for several cognitive constructs, including reinforcement learning (Frank, 2005, Cox et al., 2015), cognitive flexibility (Lee et al., 2007) and action selection, a fundamental component to self-control and inhibition (Frank, 2005, Eagle et al., 2011, Gerfen and Surmeier, 2011, Cox et al., 2015, Robertson et al., 2015).

The role of dopaminergic neurotransmission

Pharmacological studies have demonstrated a prominent role for dopamine signaling in inhibitory control. Indirect dopamine agonists increase intrasynaptic dopamine concentrations by blocking re-update of the transmitter (methylphenidate (Volkow et al., 2002a)) or facilitating release (d-amphetamine (Sulzer et al., 1995)). Administration of indirect dopamine agonists improves performance on tests of several types of inhibitory control in healthy subjects (de Wit et al., 2002, Aron et al., 2003, van Gaalen et al., 2006, Pattij and Vanderschuren, 2008, Dalley and Roiser, 2012) and improve clinical symptoms of ADHD (impulsivity, inattention) (Solanto, 2002). Similarly, dietary tyrosine supplementation increases dopamine synthesis and improves motor inhibition (Colzato et al., 2014), while dietary dopamine depletion impairs impulse control.
Taken together, these data indicate that elevating intrasynaptic dopamine concentrations improves the capacity for inhibitory control.

Pharmacological studies have indicated a selective role for D2-type receptors in inhibitory control (Pattij and Vanderschuren, 2008). The selective D2-receptor agonist, cabergoline, improves motor response inhibition (Nandam et al., 2013). Cognitive flexibility, as measured by a reversal-learning task, is modulated by administration of D2 agonist pramipexole, or antagonist amisulpride (Ersche et al., 2011). Lack of D1-specific compounds available for human use limits the investigation of the D1 receptor role in human impulsivity, however pharmacological studies in non-human primates demonstrate a specific role for D2-type, over D1-type signaling in cognitive flexibility (Lee et al., 2007).

Multi-modal neuroimaging has revealed a central role of striatal D2-type receptors in several types of inhibitory control. Striatal D2-type BP_{ND} is linked to performance on the stop-signal task (SSRT) and the inhibition-related neural activity measured with fMRI in the striatum and frontal cortex of healthy subjects (Ghahremani et al., 2012). Measures of D2-type BP_{ND} in healthy subjects is linked to neural activity related to risk-valuation during decision-making (Kohno et al., 2013). Finally, steep discounting of reward value as a function of delay, is linked to low D2-type receptor availability in the striatum of methamphetamine users (Ballard et al., 2015). Investigations of D1-type dopamine receptor function in human impulsivity are somewhat limited due to few D1-selective compounds available for human use. However, recent studies show that individual differences in striatal D1-type receptor BP_{ND} are important for motor response inhibition during Stop-signal task performance. (See Chapter 2; (Robertson et al.,
identifying D1-type signaling as a potential new target for regulating the balance between striatal output pathways, and possibly treatment for impulse control disorders.

SUMMARY OF THE DISSERTATION RESEARCH PROJECT

The exact neurobiological mechanisms that underlie inhibitory control in human subjects are still unclear. However, a central role for dopamine neurotransmission in inhibitory control is highlighted by the pharmacological effects of dopamine agonists and by neuroimaging studies investigating the neurocircuitry essential to self-control. There remains much to be learned about the connection of striatal dopaminergic signaling with various types of impulsivity and inhibitory control. Determining the relevance of individual variation in dopaminergic markers on human behavior could improve our understanding of the molecular basis of inhibitory control and its dysregulation in substance use disorders. Impulsive behavior and an inability to exert self-control over inappropriate behavior may undermine the ability to maintain abstinence and participate in treatment programs and interfere with social functioning. Therefore, improving our understanding of how neurochemistry influences impulsive behavior in human subjects is of clinical interest.

The goal of this dissertation was to increase our understanding of how striatal dopaminergic transmission via dopamine receptor subtypes (D1- and D2-type) contributes to individual differences in impulsivity and inhibitory control in healthy subjects and to investigate the extent to which dopaminergic deficits in methamphetamine users can be ameliorated with drug abstinence and behavioral treatment.
This dissertation has 3 research aims:

**Aim 1)** Determine to what extent individual variation in dopamine receptor availability (D1- and D2-type BP<sub>ND</sub>) is related to motor response inhibition capacity as estimated from neurocognitive task performance.

Using PET imaging with [<sup>11</sup>C]-NNC112 and [<sup>18</sup>F]-fallypride to assay D1-type and D2-type receptor availability (BP<sub>ND</sub>), respectively, we examined correlations with scores from two widely used neurocognitive tasks of response inhibition, the stop-signal task (SST) and continuous performance test (CPT). We examined the relationships of dopamine receptor availability (measured as binding potential relative to non-displaceable uptake; BP<sub>ND</sub>) and inhibitory control capacity, as a function of striatal anatomy (dorsal vs ventral) and receptor sub-type (D1 vs D2).

**Hypothesis:** We predicted that D1- and D2-type BP<sub>ND</sub> would be linked to indices of response inhibition capacity in opposing ways.

**Aim 2)** Examine to what extent D1- and D2-type receptor BP<sub>ND</sub> are related to scores on personality assessments of impulsivity.

Using neuroimaging techniques described above, we examined the relationships of D1- and D2-type dopamine receptor BP<sub>ND</sub> and levels of self-reported impulsiveness as assessed by scores on several widely used personality scales: The Eysenck Impulsivity Inventory (I7), Multidimensional Personality Questionnaire, (constraint MPQ), Barratt Impulsiveness Scale (BIS-11) and the Dickman Impulsivity Inventory (dysfunctional).
Hypothesis: We expected to find negative relationships between D1- and D2-type receptor $\text{BP}_{\text{ND}}$ and scores from personality scales. We predicted that D1- and D2-type receptors would have opposite associations with self-report assessments of impulsivity.

Aim 3) Dopaminergic deficits in methamphetamine use disorder are frequently reported, however but it is unknown if reversal of dopaminergic deficits occurs with drug abstinence. Therefore Aim 3 tested the extent dopaminergic deficits (D2-type $\text{BP}_{\text{ND}}$) can be ameliorated in subjects with methamphetamine use disorder.

Using individualized exercise programs, an activity known to increase dopamine system markers in rodents and humans, the effect of an 8-week exercise regimen on striatal D2-type receptors and response inhibition capacity was examined in the methamphetamine-dependent individuals undergoing behavioral treatment for their addiction in a residential treatment facility.

Hypothesis: We expected to find increases in D2-type receptor $\text{BP}_{\text{ND}}$ in the exercise condition but not in subjects in the education-control condition.
References for Chapter 1


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CHAPTER 2: STRIATAL D1- AND D2-TYPE RECEPTORS ARE LINKED WITH MOTOR RESPONSE INHIBITION IN HUMAN SUBJECTS

THIS CHAPTER IS A VERSION OF A PUBLISHED MANUSCRIPT:

Neural circuits involving dopaminergic transmission mediate motor response inhibition; however, the relative contributions of dopaminergic signaling via D1- and D2-type receptors are unclear. Although evidence supports dissociable contributions of D1- and D2-type receptors to response inhibition in rats and associations of D2-type receptors to response inhibition in humans, the relationship between D1-type receptors and response inhibition has not been evaluated in humans. Here, we tested whether individual differences in striatal D1- and D2-type receptors are related to response inhibition in human subjects, possibly in opposing ways.

Thirty-one volunteers participated. Response inhibition was indexed by stop-signal reaction time on the Stop-Signal Task and commission errors on the Continuous Performance Task, and tested for association with striatal D1- and D2-type receptor availability (binding potential, $BP_{ND}$), measured using positron emission tomography with $[^{11}\text{C}]$NNC-112 and $[^{18}\text{F}]$fallypride, respectively. Stop-signal reaction time was negatively correlated with D1- and D2-type $BP_{ND}$ in whole striatum, with significant relationships involving the dorsal but not ventral striatum, and no significant correlations involving the Continuous Performance Task.

The results indicate that dopamine D1- and D2-type receptors are associated with response inhibition, and identify the dorsal striatum as an important locus of dopaminergic control in stopping. Moreover, the similar contribution of both receptor subtypes suggests the importance of a relative balance between phasic and tonic dopaminergic activity subserved by D1- and D2-type receptors, respectively, in support of response inhibition. The results also suggest that the Stop-Signal Task and the Continuous Performance Task utilize different neurochemical mechanisms subserving motor response inhibition.
INTRODUCTION

Impulsive actions are premature, poorly conceived, or difficult to suppress (Dalley et al., 2008), and lack of inhibitory control over impulsiveness is a hallmark of attention deficit hyperactivity disorder (ADHD) and substance-use disorders (Bari and Robbins, 2013). Impaired inhibitory control can disrupt goal-directed behavior, with negative consequences that contribute to psychological distress associated with these disorders. Clarifying the mechanisms that mediate inhibitory control, therefore, ultimately may help guide treatment for disorders characterized by an impulsive phenotype.

Research findings have indicated a role of dopamine in impulsive behavior. Syndromes, such as ADHD (Vaidya et al., 1998, Bedard et al., 2003, Senderecka et al., 2012) and addictions (Fillmore and Rush, 2002, Monterosso, 2005, Lane et al., 2007, Lee et al., 2009), which feature behavioral disinhibition, are associated with dopaminergic dysfunction. In addition, studies of genetic polymorphisms (Colzato et al., 2010, Colzato et al., 2013) and pharmacological manipulations (Chamberlain et al., 2006, Eagle and Baunez, 2010) have identified a role for dopaminergic signaling in motor response inhibition, an index of inhibitory control (Chamberlain et al., 2006, Eagle and Baunez, 2010). For example, methylphenidate or d-amphetamine administration improves response inhibition in ADHD patients and healthy subjects, respectively (Tannock et al., 1989, de Wit et al., 2000, Aron et al., 2003), and manipulation of dietary tyrosine (dopamine precursor) alters response inhibition (Colzato et al., 2014, Ramdani et al., 2014).

Despite an evident role for dopamine, the relative contributions of dopamine signaling via dopamine D1- and D2-like receptor subtypes are unclear. In rats, systemic administration of
dopaminergic antagonists does not affect response inhibition (Eagle et al., 2007, Eagle et al., 2008, Bari and Robbins, 2013). However, direct infusion of the D1-receptor antagonist, SCH-23390, into the dorsal-medial striatum, improves response inhibition, whereas infusion of the D2 receptor antagonist sulpiride has the opposite effect. Similar infusions into the ventral striatum have no effect (Eagle et al., 2011). Thus, the effects of dopamine-receptor-subtype signaling on response inhibition appear to be regionally specific and possibly opposing. In addition, administration of the D2-specific agonist, cabergoline, improves response inhibition (Nandam et al., 2013), and striatal D2-type receptor availability is correlated with the capacity for response inhibition and corresponding neural activation during inhibition in humans (Ghahremani et al., 2012). Nonetheless, human investigations of the role of D1-type receptors in response inhibition and direct comparisons of D1- vs D2-type receptor contributions to motor response inhibition have not been performed.

We used positron emission tomography (PET) with $[^{11}\text{C}]$NNC-112 and $[^{18}\text{F}]$fallypride as radioligands for dopamine D1- and D2-type receptors, respectively (Mukherjee et al., 1995, Ekelund et al., 2007), to examine the relationships of subtype-selective dopamine-receptor availability ($\text{BP}_{\text{ND}}$) with measures from prototypical assessments of motor response inhibition — the Stop-Signal Task (SST) and Continuous Performance Task (CPT) (Logan et al., 1984, Tannock et al., 1989, Aron et al., 2014). We hypothesized that dopamine receptors in the dorsal, but not the ventral, striatum would be linked to response-inhibition task performance, and that D1- and D2-type receptor contributions would be dissociable in this region, reflecting opposing actions.
MATERIALS AND METHODS

Research Participants

All study procedures were approved by the University of California Los Angeles (UCLA) Institution Review Board. Thirty-one healthy volunteers (16 female, mean age = 30.68 years, SD = 8.3), participating in the UCLA Consortium for Neuropsychiatric Phenomics (CNP; www.phenomics.ucla.edu), completing extensive neuropsychological testing, including tests of response inhibition, and MRI scanning (Bilder et al., 2009). CNP participants who expressed interest in being contacted for additional studies were offered flyers or were called via telephone, and were invited to participate in this study, involving PET scanning. On average, PET scanning occurred approximately 17 months after participation in the CNP study. Participants received a complete description of this study, and provided written informed consent. Health screening was performed using the Structured Clinical Interview for DSM-IV and a physical examination.

Participants were excluded if they met the following criteria: current axis I psychiatric diagnoses other than nicotine dependence; use of psychotropic medications or substances, except marijuana or alcohol; CNS, cardiovascular or systematic disease; HIV seropositive status, hepatic disease or pregnancy. On all test days, negative urine samples for recent drug use and pregnancy (women) were required.

Neurobehavioral tasks

The Stop-Signal Task (SST) (Logan et al., 1984) and Continuous Performance Task (CPT) (Tannock et al., 1989) were administered via PC laptop (E-prime 2.0, Psychology Software Tools). During the SST, participants viewed a series of go stimuli (left/right arrows) and were instructed to respond with corresponding left or right key presses, respectively (go trials). On some trials (stop trials, 25%), an audible tone (stop-signal) was presented after a short
delay (stop-signal delay, SSD) following the go stimulus. Participants were instructed to withhold their responses upon hearing the tone. They were instructed to respond quickly and accurately, and that stopping and going were equally important. The SSD was adjusted on a trial-by-trial basis according to performance; values were drawn from two interleaved ladders to ensure equal performance level across participants, producing successful inhibition on ~50% of stop trials. Participants received task training prior to task initiation, consisting of eight trials (three of which were stop trials).

During the CPT, participants viewed a series of go stimuli (alphabet letters, go trials), and were instructed to respond with a key press. On some trials (no-go trials, 10%) a no-go stimulus was presented (the letter “X”) in lieu of the go stimulus, and participants were instructed to withhold responding. The task comprised 18 blocks presented at random, each containing 20 trials at a fixed inter-trial interval (ITIs): 1000, 2000, or 4000ms. Participants received task training prior to initiation, consisting of 10 trials from the 2000-ms ITI type.

Analysis of neurobehavioral data

SST data were analyzed using the same methods as in a prior study of a separate sample (Ghahremani et al., 2012). The median and standard deviation of reaction time on go trials were calculated using all-correct go trials (GoRT). The average SSD was calculated using all-successful stop trials. The stop-signal reaction time (SSRT) was estimated by subtracting each participant’s average SSD from his/her median GoRT (Band et al., 2003). Percent inhibition on stop trials was calculated as the ratio of successful stop trials to all stop trials presented. As recommended (Congdon et al., 2012), participant data meeting the following criteria were removed from analysis: (1) < 25% or > 75% inhibition on stop trials (n=3), (2) < 60% correct responding on go trials (n=0), (3) > 10% direction errors on go trials (n=0), (4) SSRT estimate
that was negative or <50 ms (n=1, computer failure). SST data from 27 participants were subject to analysis: 22 with D1-type BP_{ND}, 24 with D2-type BP_{ND}, and 19 with both. Performance data from the CPT were used to calculate the mean and standard deviation of go-trial reaction time (GoRT) on all go trials. Commission error (CE) was calculated as the number of failed no-go trials (response to a no-go stimulus).

**PET scanning**

D1-type dopamine receptor availability (D1-type BP_{ND}) was assayed using \([^{11}C]NNC-112\), a high-affinity ligand for D1-type receptors (Andersen et al., 1992, Ekelund et al., 2007), in 26 subjects (14 female). Dopamine D2-type receptor availability (D2-type BP_{ND}) was assayed on a different day using \([^{18}F]fallypride\), a high affinity radioligand for D2-type receptors (Mukherjee et al., 1995) in 27 subjects (14 female). PET scanning was performed on a Philips Gemini Tru Flight PET/CT in 3D mode (Philips Electronics, Netherlands; FWHM = 5.0 mm × 4.8 mm, 90 slices, voxel size 2 mm^3). A CT-transmission scan was performed to obtain data for measured attenuation correction. After bolus injection of \([^{11}C]NNC-112\) (~15 mCi ±5%, specific activity ≥ 1 Ci/μmol), dynamic emission data were acquired for 90 min. For \([^{18}F]fallypride\) (~5 mCi ±5%, specific activity ≥ 1 Ci/μmol), data were acquired in two scanning blocks of 80-min each, with a short break between blocks. Data were reconstructed using the 3D row action maximum likelihood algorithm (3D-RAMLA). Scatter and random corrections were applied.

**PET image processing**

Reconstructed \([^{11}C]NNC-112\) PET data (1-min × 90-frames) were averaged into 23 frames, consisting of four 1-min frames, three 2-min frames and sixteen 5-min frames. Reconstructed \([^{18}F]fallypride\) PET data (2 blocks; 1-min × 80-frames) were combined into 16
frames, each consisting of the average of 10-min. PET images were motion-corrected (Jenkinson et al., 2002) then co-registered to the corresponding MRI (Jenkinson and Smith, 2001). VOI-based time-activity data were extracted for kinetic modeling using PMOD (PMOD 3.1, Zurich). Time-activity curves were fit using the simplified reference tissue model (SRTM)(Lammertsma and Hume, 1996). The cerebellum was selected as the reference region (Hall et al., 1994, Abi-Dargham et al., 2000, Ishibashi et al., 2013). A volume-weighted average of k2', estimated from high-activity regions (caudate and putamen), was computed. Time-activity curves were then refit using SRTM2 (Wu and Carson, 2002), applying the computed k2' values to all VOIs. Binding potential referred to non-displaceable uptake (BPND) was calculated by subtracting 1.0 from the product of R1 and k2'/k2a.

MRI scanning and volumes of interest (VOIs)

MRI scanning was performed on a Siemens Trio (MPRAGE: repetition time = 1.9 sec, echo time = 2.26 msec, voxel size = 1 mm³, 176 slices), and processed using the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/index.html, Oxford University).

Selected VOIs included the whole striatum and functional striatal subdivisions: limbic striatum, associative striatum, and sensory-motor striatum. A VOI for the whole striatum was created by combining anatomically defined VOIs for the caudate, putamen and nucleus accumbens using the FSL software package (Patenaude et al., 2011). Functional subdivisions of the striatum (Mawlawi et al., 2001) and the midbrain region (Zald et al., 2010) were defined as described previously. The cerebellum VOI was drawn manually in standard space and transformed to each subject’s MRI.
Data analysis and statistical analysis

Striatal VOIs were selected *a priori* on the basis of evidence that dopaminergic transmission in these regions is important for inhibitory control (Lee et al., 2009, Buckholtz et al., 2010, Ghahremani et al., 2012). Correlations of BP\(_{ND}\) with behavioral measures in striatal functional subdivisions were tested *post-hoc* if a significant relationship was found using the whole-striatum VOI. Relationships between BP\(_{ND}\), SST, and CPT were conducted analyzed using SPSS (SPSS 22; IBM Corp., Armonk, NY). Analyses reported here were conducted using measurements from bilateral VOIs. These correlations were nearly identical to those examined using measurements from left and right VOIs separately. Exploratory investigations of D1- and D2-type BP\(_{ND}\) with SST and CPT performance included a voxel-wise analysis of correlations between cortical BP\(_{ND}\) and SSRT, GoRT or CE and a VOI-based analysis using measurements of midbrain BP\(_{ND}\).

Relationships of regional D1-type and D2-type BP\(_{ND}\)

Within-region correlations of D1- and D2-type BP\(_{ND}\) were performed for all striatal VOIs by Pearson correlation analysis. Of the 31 participants included in the study, 22 (12 female) underwent PET scans for determination of both D1- and D2-type BP\(_{ND}\), and their data were used for this analysis.

Dopamine receptor BP\(_{ND}\) and performance on the SST and CPT

Relationships of striatal BP\(_{ND}\) with SSRT were tested using partial correlation analysis controlling for age and sex. Similar analyses were preformed with GoRT.
The Hotelling-Williams test (Van Sickle, 2003) was used to test for equality of the correlations between SSRT and dorsal striatum BP\textsubscript{ND} versus SSRT and ventral striatum BP\textsubscript{ND}. For this test, BP\textsubscript{ND} values of the associative and sensory-motor regions were combined to create a BP\textsubscript{ND} value for the dorsal striatum, and compared to the BP\textsubscript{ND} value of the ventral striatum.

To examine the contributions of both receptor subtypes (BP\textsubscript{ND}) on SSRT, a step-wise regression analysis was used. To determine the effect of adding D2-type BP\textsubscript{ND} to a model with D1-type BP\textsubscript{ND}, the variables entered into the first step of the regression were age, sex and D1-type BP\textsubscript{ND}. D2-type BP\textsubscript{ND} was included in the second step. Next, the reverse relationship was tested, to determine the effect of adding D1-type BP\textsubscript{ND} to a model using D2-type BP\textsubscript{ND}, with D1-type BP\textsubscript{ND} included in the second step instead.

The relationships of receptor BP\textsubscript{ND} with commission errors (CE), the outcome variable reflecting response inhibition in the CPT, and GoRT were assessed using partial correlation analysis, controlling for sex and age.

To estimate effects of time lapse between neuroimaging and neurobehavioral procedures (average 17 months), the stability of neurobehavioral task performance over time was evaluated. For this analysis, a subset of participants (n=10) was invited to return for re-testing of SST and CPT performance after an average elapsed time of 40 months. Reliability assessments were assessed using the intraclass correlation coefficient (ICC).
RESULTS

Neurobehavioral tasks (Table 1)

On the Stop-Signal Task, participants performed at a level of 99% correct on go trials and inhibited their responses on approximately half of the stop trials [mean (SD): 52% (0.056)], indicating that the adaptive staircase procedure for equating stop-trial performance across participants was successful. SSRT and values were similar to those observed in prior studies of separate samples (Boehler et al., 2010, Ghahremani et al., 2012). On the CPT, participants averaged 99% correct on go trials and averaged 13 CE (of 36 no-go trials). Mean GoRT values were similar to those reported previously (Steele et al., 2013).

Dopamine receptor BP\textsubscript{ND} (Table 2, Figure 4)

Overall, BP\textsubscript{ND} values for both receptor subtypes were higher in the dorsal than in ventral regions of the striatum. D1- and D2-type BP\textsubscript{ND} values were approximately equal in the associative and sensory-motor striatum, while D2-type BP\textsubscript{ND} was higher in the sensory-motor than the associative striatum. D1-type receptor BP\textsubscript{ND} and D2-type BP\textsubscript{ND} showed no significant correlation in the associative or limbic striatal subdivisions, but were significantly positively correlated in the sensory-motor striatum ($r = 0.469, p = 0.028$).

Dopamine receptor BP\textsubscript{ND} and response inhibition on the SST (Table 3)

SSRT was negatively correlated with D1-type BP\textsubscript{ND} in the whole striatum, controlling for the effects of age and sex ($r = -0.624, p = 0.003$)(Figure 1). Post-hoc evaluations of data from functional subdivisions of the striatum revealed significant relationships in the dorsal regions (associative striatum: $r = -0.548, p = 0.012$; sensory-motor striatum: $r = -0.527, p = 0.017$) but not in the ventral region (limbic striatum: $r = -0.342, p = 0.139$). A difference in correlations
between SSRT and D1-type BP<sub>ND</sub> in the dorsal vs. ventral region of striatum was detected using the Hotelling-Williams test at a trend level (p = 0.083). To determine the specificity of the association to the stopping process, correlations between GoRT and D1-type BP<sub>ND</sub> were examined. D1-type BP<sub>ND</sub> in the whole striatum showed a trend towards negative correlation with GoRT (r = -0.425, p = 0.062). We therefore conducted post-hoc analysis of the functional subdivisions, and found that the correlation involving D1-type BP<sub>ND</sub> in the ventral striatum reached a trend level (p = 0.082), but the Hotelling-Williams test indicated no difference in dorsal versus ventral correlations.

SSRT was negatively correlated with D2-type BP<sub>ND</sub> in the whole striatum, controlling for the effects of age and sex (r = -0.478, p = 0.021)(Figure 1). Post-hoc tests involving functional subdivisions of the striatum showed significant negative correlations in the associative striatum (r = -0.544, p = 0.007) and sensory-motor striatum (r = -0.419, p = 0.046) but not in the limbic striatum (r = -0.308, p = 0.153)(Table 3). The Hotelling-Williams test of equality of correlations showed that the relationships of SSRT and D2-type BP<sub>ND</sub> in the dorsal versus ventral regions of striatum differed significantly from one another (p = 0.039), suggesting that the correlation of D2-type BP<sub>ND</sub> with SSRT was specific to the dorsal striatum.

Since BP<sub>ND</sub> for each receptor subtype in the dorsal striatum was negatively correlated with SSRT, we tested the correlations between BP<sub>ND</sub> for each receptor subtype and SSRT, controlling for the effects of age, sex and BP<sub>ND</sub> for the other receptor subtype. A negative correlation of SSRT with D1-type BP<sub>ND</sub> in the associative striatum was present at trend-level when controlling for D2-type BP<sub>ND</sub> (r = -0.473, p = 0.064). SSRT was negatively correlated with D2-type BP<sub>ND</sub> in associative striatum when controlling for D1-type BP<sub>ND</sub> (r = -0.599, p = 0.014).
To examine the effect both receptor BP\textsubscript{ND} on SSRT, a stepwise regression was used to determine the effect of adding additional BP\textsubscript{ND} measures to a model of SST performance using only one BP\textsubscript{ND} measure. A model using age, sex and D1-type BP\textsubscript{ND} to predict SST performance was improved by adding D2-type BP\textsubscript{ND} to the model (D1: F\textsubscript{3,18} = 2.937, p=0.067)(D1+D2: F\textsubscript{4,18} = 3.776, p=0.028). In the reverse analysis, adding D1-type BP\textsubscript{ND} to a model of SST performance using D2-type BP\textsubscript{ND} also improved the model (D2: F\textsubscript{3,18} = 2.176, p=0.133)(D1+D2: F\textsubscript{4,18} = 3.776, p=0.028). The model including both receptors showed main effects of both D1- and D2-type BP\textsubscript{ND} (D1: t= -2.506, p = 0.025; D2: t= -2.082, p = 0.056).

**Dopamine receptor BP\textsubscript{ND} and response inhibition assessed by the CPT**

Tests of the correlations between dopamine receptor subtype BP\textsubscript{ND} and commission error (CE) or GoRT on the CPT showed no statistically significant relationships. Furthermore, CE was not correlated with response inhibition capacity (SSRT) on the SST. Although GoRT on the SST and GoRT on the CPT showed a significant association (r = 0.419, p = 0.024), GoRT on the CPT did not show any significant relationship with either D1- or D2-type BP\textsubscript{ND} in any region tested.

Neither D1- nor D2-type BP\textsubscript{ND} in the cortex showed a significant correlation with SSRT, GoRT or CE in a voxel-wise analysis, using a liberal threshold (p < 0.05, uncorrected). Analysis of D1- or D2-type BP\textsubscript{ND} in the midbrain showed no significant correlations with SSRT, GoRT or CE.

**Repeated measures of neurobehaviorial task performance**

Task-performance variables showed a high degree of test-retest reliability over an average elapsed time of 40 months. Average percent change in CE and SSRT was small (8%, 7% respectively), and intraclass correlations were moderately high (CE: ICC = 0.913, p = 0.001,
n=10; SSRT: ICC = 0.738, p = 0.029, n=10). Adding the time interval between neuroimaging and neurocognitive tests as a covariate in statistical analyses did not change the results.

**DISCUSSION**

This study extends evidence for a contribution of striatal dopaminergic function to motor response inhibition in humans (Ghahremani et al., 2012, Bari and Robbins, 2013, Nandam et al., 2013), demonstrating involvement of both D1- and D2-type dopamine receptors in the dorsal striatum. D1 and D2 receptors are localized to striato-nigral and striato-pallidal neurons, respectively, with minimal co-localization (Hersch et al., 1995). Dopamine regulates striatal activation and output via D1-receptor activation, which enhances the function of striato-nigral neurons, and via D2-receptor activation, which suppresses function of striato-pallidal neurons (Creese et al., 1983, Surmeier et al., 2007, Gerfen and Surmeier, 2011). Dopamine can activate both D1- and D2-type receptors, but the relative activation of either subtype depends on intrasynaptic dopamine concentration and the respective affinities of the receptors for the neurotransmitter. D2-type receptors, which have higher affinity than D1-type receptors for dopamine, mediate tonic dopaminergic signaling. D1-type receptors are activated at high dopamine concentrations, during phasic increases in extracellular dopamine (Dreyer et al., 2010). D1- and D2-type receptor signaling can have synergistic effects, as shown by the observation that co-administration of D1- and D2-type dopamine receptor agonists, at doses that are behaviorally inactive when administered alone, increases locomotor behavior in rats (Vermeulen et al., 1994). Such an interaction between striatal D1- and D2-modulated pathways may govern performance on the SST.
The results obtained here align with a model of striatal motor control of response inhibition in which D1- and D2-type receptors support competing processes via the modulation of the go (striato-nigral) and no-go (striato-pallidal) pathways (Logan et al., 1984, Mink, 1996, Frank, 2005, Frank et al., 2007). This model posits that D1-expressing striato-nigral neurons facilitate the “go” process and D2-expressing striato-pallidal neurons facilitate the “stop” process (Alexander and Crutcher, 1990, Surmeier et al., 2007, Gerfen and Surmeier, 2011). The negative correlation observed here between D2-type BP_{ND} and SSRT is consistent with this model and corroborates findings from other human studies showing that administration of the D2-type receptor agonist, carbergoline, enhances stopping ability (Nandam et al., 2013) and from neuroimaging results showing that striatal D2-type BP_{ND} is correlated with SSRT and inhibition-related striatal neural activity, measured with fMRI (Ghahremani et al., 2012).

Several studies have described opposing contributions of D1- and D2-mediated dopamine signaling to cognitive function and behavior. For example, individual differences in the ability to learn from positive and negative feedback are related to D1- and D2-type BP_{ND} values, respectively (Cox et al., 2015). A theory of prefrontal dopamine function describes a balance between D1- and D2-type receptor-mediated signaling in modulating fronto-striatal function (Durstewitz and Seamans, 2008). Moreover, a new model of dopamine function in the basal ganglia posit that D1 receptor activation prepares a set of possible responses, then D2 receptor activation functions in shaping and selecting the final response (Keeler et al., 2014). The present findings are consistent with such integrated function, suggesting that there is cooperative signaling between D1- and D2-type receptor-mediated pathways during stopping.
The effect of D2-type BP$_{ND}$ on SSRT appears to be specific to stopping a motor response, as indicated by the lack of correlation with GoRT. In contrast, the relationship between D1-type BP$_{ND}$ and SSRT may reflect a general motor effect. This view is supported by the trend-level correlation found with GoRT on the SST, and by literature showing consistently that activation of D1 receptors enhances motor activity (Kreitzer and Berke, 2011). D1-type BP$_{ND}$, however, was not correlated with GoRT on the CPT.

The anatomical specificity of the correlations between SSRT and BP$_{ND}$ corroborate findings from rodent studies using excitotoxic lesions (Eagle and Robbins, 2003b) and pharmacological manipulations (Eagle et al., 2011). These studies showed that dopaminergic transmission in the dorsal but not the ventral striatum is necessary for SST performance. Specifically, neither excitotoxic lesions nor direct antagonist infusions into the nucleus accumbens affected SST performance in rats (Eagle and Robbins, 2003b, a, Eagle et al., 2011). Moreover, this uniquely dorsal striatal relationship with SST performance was also observed in humans in which D2-type BP$_{ND}$ and fMRI activation during stopping was found in dorsal but not ventral striatum (Ghahremani et al., 2012). Lastly, although D2-type BP$_{ND}$ in the midbrain has been associated with self-reports of impulsivity and novelty-seeking (Zald et al., 2008, Buckholtz et al., 2010), there were no significant relationships between the behavioral performance measures and D2-type BP$_{ND}$ in the midbrain. This difference between findings may reflect differences between what is measured by self-reports of impulsivity as compared with neurocognitive tasks (Reynolds et al., 2006, Reynolds et al., 2008, Fields et al., 2009).

That performance on the SST but not the CPT was associated with dopamine receptor availability suggests that the tasks tap into different neurochemical mechanisms subserving
motor response inhibition. Whereas the SST measures the ability to cancel a motor response that has been initiated, the CPT measures action restraint (i.e., not going). Brain-imaging studies have shown that these tasks engage overlapping, but distinct, neural circuits (Rubia et al., 2001, Zheng et al., 2008, Swick et al., 2011, Steele et al., 2013). If the SST and CPT were identical measures of response inhibition, they would be governed by the same neurotransmitter systems, and show comparable relationships with neurochemical markers (Jentsch et al., 2014). Our findings, however, support a functional distinction between stopping (SST) and not going (CPT) as separate constructs (Robinson et al., 2009, Swick et al., 2011) that are subserved by different neurochemical substrates (Dalley et al., 2008, Robinson et al., 2009). These results suggest that, whereas the latency of the inhibition process (SSRT) is likely influenced by dopaminergic signaling, the ability to withhold a response (CPT) is not (Eagle and Baunez, 2010). Different cognitive requirements, such as those involving attention or working memory, may influence overall task performance and links to dopamine markers. Such differences may also explain the lack of correlation between scores on the CPT and SST in both rodents and human subjects (Broos et al., 2012). Finally, while dopamine receptors were the main focus of this study, contributions of other neurotransmitter systems cannot be overlooked, as there is substantial evidence for a role of noradrenergic and other transmitter systems in the striatal control of response inhibition (Zheng et al., 1999, Eagle et al., 2011, Bari and Robbins, 2013).

This study has limitations. Among them are its correlative design, which cannot inform on causal relationships between dopamine-receptor subtype signaling and motor response inhibition, and the relatively small sample size. Another is imperfect selectivity of the radioligands used. [$^{11}$C]NNC-112 has approximately 10-fold higher \textit{in vivo} affinity for D1-type over 5HT2A receptors (Slifstein et al., 2007), and pharmacological blocking studies show that
~5% of the $[^{11}\text{C}]$NNC-112 signal in the striatum represents 5HT2A binding (Ekelund et al., 2007). Although contamination of the D1-receptor signal with 5HT2A binding is minor in the striatum, it should be acknowledged. $[^{18}\text{F}]$fallypride has nearly equal affinity for D2 and D3 dopamine receptors in vivo (Slifstein et al., 2004) and cannot distinguish between them; however, D2-type receptors in the dorsal striatum are almost exclusively D2 receptors with very low D3 expression (Murray et al., 1994). Thus, BP$_{\text{ND}}$ measurements in the dorsal striatum primarily reflect D2 receptor availability, and those in the ventral striatum are likely a combination of signal from D2 and D3 receptors. $[^{18}\text{F}]$fallypride also binds to both isoforms of the D2 receptor (D2S and D2L); therefore, BP$_{\text{ND}}$ measurements using $[^{18}\text{F}]$fallypride do not distinguish between pre- and post-synaptic D2 receptors.

Another limitation is the time interval between the behavioral and PET assessments, which was 17 months on average. Of relevance is the low test-retest variation in BP$_{\text{ND}}$ measurements made using $[^{11}\text{C}]$NNC-112 or $[^{18}\text{F}]$fallypride, which has been determined in previous studies to be 5-10% (Abi-Dargham et al., 2000, Fujita et al., 2006, Dunn et al., 2013), and the small change in D1- and D2-type BP$_{\text{ND}}$ with aging, a decrease of only ~ 8% with every decade of life. In addition, test-retest reliability of the SST and CPT performance variables is well established, showing high reliability over several weeks (Soreni et al., 2009, Weafer et al., 2013) but also see (Wostmann et al., 2013) and high reliability of performance on both of the neurobehavioral tasks, with an average elapsed time of 40 months between assessments. Adding the time interval between neuroimaging and neurocognitive tests as a covariate in statistical analyses did not change the results, suggesting that the time-related influences on the relationships between dopamine receptor BP$_{\text{ND}}$ and task performance reported here are likely to be minimal.
In summary, we present direct evidence for associations of striatal D1- and D2-type receptor availability with capacity for response inhibition on the SST in humans. These relationships were specific to the dorsal striatum, identifying this region as an important locus for differential dopaminergic control of motor response inhibition. The results support the notion that the balance between D1- and D2-type receptor mediated signaling is important for motor response inhibition. The findings represent an important advance as the understanding of dopaminergic signaling in the human brain has implications for the development of specific agents, possibly D1-targeted, to treat patients with neuropsychiatric disorders that are characterized by an impulsive phenotype, such as observed in ADHD and addictive disorders.
**Figures**

Figure 2.1 - Scatter plot depicting the correlation between stop-signal reaction time (SSRT), and D1-type receptor binding potential (D1-type BP<sub>ND</sub>) in the whole striatum. Table insert displays partial correlation coefficients, p values and R<sup>2</sup> for the relationship between whole striatum and associative striatum D1-type BP<sub>ND</sub> and SSRT, controlling for age and sex.
Figure 2.2: Scatter plot depicting the correlation between stop-signal reaction time (SSRT), and D2-type receptor binding potential (D2-type BP\textsubscript{ND}) in whole striatum. Table insert displays partial correlation coefficients, p values and $R^2$ for the relationship between whole striatum and associative striatum D2-type BP\textsubscript{ND} and SSRT, controlling for age and sex.
Figure 2.3: Voxel-wise effect size maps depicting the partial correlation coefficient (r) between individual stop-signal reaction time (SSRT) and D1-type (A) and D2-type (B) receptor binding potential (BP\textsubscript{ND}) in the striatum, controlling for the effects of age and sex.
Figure 2.4: Scatter plot depicting the relationship between D2-type receptor binding potential (D2-type BP\textsubscript{ND}) and D1-type receptor binding potential (D1-type BP\textsubscript{ND}) in whole striatum, z-scores of BP\textsubscript{ND} were used for presentation purposes. Table insert displays correlation coefficients, p-values and R\textsuperscript{2} for the correlations in the whole and associative striatum.
Table 2.1: Performance variables for the Stop-Signal Task and the Continuous Performance Task.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stop-signal task</strong> ($n = 27$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRT (ms)</td>
<td>235</td>
<td>37</td>
</tr>
<tr>
<td>Median GoRT (ms)</td>
<td>567</td>
<td>109</td>
</tr>
<tr>
<td>SD GoRT (ms)</td>
<td>112</td>
<td>30</td>
</tr>
<tr>
<td>Correct go responding (%)</td>
<td>99</td>
<td>0.007</td>
</tr>
<tr>
<td>Inhibition on stop-trials (%)</td>
<td>52</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>Continuous performance task</strong> ($n = 31$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean GoRT (ms)</td>
<td>372</td>
<td>45</td>
</tr>
<tr>
<td>Median GoRT (ms)</td>
<td>356</td>
<td>39</td>
</tr>
<tr>
<td>SD of GoRT (ms)</td>
<td>85</td>
<td>25</td>
</tr>
<tr>
<td>Commission errors</td>
<td>13</td>
<td>5.9</td>
</tr>
</tbody>
</table>
Table 2.2: Means and standard deviations of D1-type and D2-type binding potential (BP\textsubscript{ND}) in the striatum and within-region correlations. *p = 0.028. n= 22 (12 females).

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>D\textsubscript{1}-type BP\textsubscript{ND}</th>
<th>D\textsubscript{2}-type BP\textsubscript{ND}</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole striatum</td>
<td>1.98 (0.19)</td>
<td>29.59 (4.68)</td>
<td>0.310</td>
</tr>
<tr>
<td>Limbic striatum</td>
<td>1.82 (0.18)</td>
<td>26.38 (4.26)</td>
<td>0.193</td>
</tr>
<tr>
<td>Associative striatum</td>
<td>2.02 (0.23)</td>
<td>29.29 (4.55)</td>
<td>0.259</td>
</tr>
<tr>
<td>Sensory motor striatum</td>
<td>2.01 (0.21)</td>
<td>31.87 (5.77)</td>
<td>0.469*</td>
</tr>
</tbody>
</table>

Data are reported as the mean (SD).
Table 2.3: Correlation coefficients and p-values for the relationships of dopamine receptor binding potential ($BP_{ND}$) and Stop-Signal task performance variables, controlling for the effects of age and sex. Bold font indicates statistical significance.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>SSRT</th>
<th>GoRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>$D_1$-type $BP_{ND}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole striatum</td>
<td>-0.624</td>
<td>0.003</td>
</tr>
<tr>
<td>Limbic striatum</td>
<td>-0.342</td>
<td>0.139</td>
</tr>
<tr>
<td>Associative striatum</td>
<td>-0.548</td>
<td>0.012</td>
</tr>
<tr>
<td>Sensory motor striatum</td>
<td>-0.527</td>
<td>0.017</td>
</tr>
<tr>
<td>Midbrain</td>
<td>-0.373</td>
<td>0.106</td>
</tr>
<tr>
<td>$D_2$-type $BP_{ND}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole striatum</td>
<td>-0.478</td>
<td>0.021</td>
</tr>
<tr>
<td>Limbic striatum</td>
<td>-0.308</td>
<td>0.153</td>
</tr>
<tr>
<td>Associative striatum</td>
<td>-0.544</td>
<td>0.007</td>
</tr>
<tr>
<td>Sensory motor striatum</td>
<td>-0.419</td>
<td>0.046</td>
</tr>
<tr>
<td>Midbrain</td>
<td>-0.327</td>
<td>0.137</td>
</tr>
</tbody>
</table>
References for Chapter 2


Bari A, Robbins TW (2013) Noradrenergic versus dopaminergic modulation of impulsivity, attention and monitoring behaviour in rats performing the stop-signal task: possible relevance to ADHD. Psychopharmacology (Berl) 230:89-111.


Eagle DM, Robbins TW (2003b) Lesions of the medial prefrontal cortex or nucleus accumbens core do not impair inhibitory control in rats performing a stop-signal reaction time task. Behav Brain Res 146:131-144.


CHAPTER 3: PERSONALITY SCALES OF IMPULSIVENESS: RELATIONSHIPS TO STRIATAL D1- AND D2-TYPE RECEPTORS.
INTRODUCTION

Impulsivity, as a broadly defined concept, encompasses several definitions that describe a general tendency to act without thinking and engage in impulsive behaviors. Impulsive behaviors are carried out without forethought or regard to potential consequences, or are unduly risky or inappropriate (Evenden, 1999, Dalley and Roiser, 2012). Several methods were developed to measure and quantify impulsivity, including self-report personality scales and neurobehavioral tasks of inhibition. These methods have different benefits and shortcomings associated with their use. Personality scales ask the subject to rate the degree to which they endorse a series of statements describing impulsive behavior i.e. “I make decisions quickly,” “I often buy things I cannot afford,” or “I act without thinking” (Evenden, 1999). These assessments are considered to be more “trait-like” in nature, and remain relatively stable over time and across biological conditions (de Wit, 2009, Gullo and Potenza, 2014). However, impression management and lack of insight on one’s own behavior contribute to measurement variability and noise in the data (Pattij and Vanderschuren, 2008). Neurobehavioral tasks, on the other hand, are intended to provide an objective measure of variation in impulsivity. Performance on neurobehavioral tasks are considered to be “state-like,” meaning that task performance is likely influenced by emotional and biological conditions (Cyders and Coskunpinar, 2011).

Both methods are used in substance abuse research and demonstrate heightened impulsiveness is associated with stimulant use disorders (Semple et al., 2005, de Wit, 2009). The high degree of overlap between substance abuse and impulsivity suggests that they operate within a similar set of neurobiological processes (Jentsch and Taylor, 1999, de Wit, 2009, Trifilieff and Martinez, 2014), and several lines of evidence suggest dopaminergic transmission within the corticostriatal circuitry to be involved in several types of impulsive behaviors and their subsequent control (Jentsch and Taylor, 1999, de Wit, 2009, Trifilieff and Martinez, 2014).
Individual variation in scores from both self-report and behavioral-tasks show associations with striatal dopaminergic markers (Cools et al., 2009), especially D2-type receptors (Lee et al., 2009, Buckholtz et al., 2010, Ghahremani et al., 2012, Robertson et al., 2015). These findings suggest the possibility that the constructs measured with self-report and neurocognitive assessments of impulsiveness may recruit similar neurocircuitry.

In the last chapter (Study 1), the study demonstrated a link between D1- and D2-type dopaminergic receptors in the striatum and performance on two neurobehavioral tasks of motor response inhibition. These results are important because they identify a role for dopaminergic signaling in both the direct and indirect pathway striatal pathways. This broadens our conventional understanding of inhibitory control is as predominantly D2-related (indirect pathway) process, to include a role for dopamine transmission in the direct pathway via D1-type receptors as well.

Previous studies show that self-reported impulsiveness is linked to D2-type receptors in the striatum (Lee et al., 2009, Buckholtz et al., 2010), the findings from Study 1 (Chapter 2) (Robertson et al., 2015) suggest that impulsiveness may also relay on signaling through the direct and indirect pathways, and would show similar relationships with D1- and D2-type receptors in the striatum. While the relative contributions of D1 and D2 type receptors to cognitive functions have been examined (Karlsson et al., 2011, Takahashi et al., 2012), these have not been examined in personality assessments of impulsiveness. Investigation of the role of dopamine in impulsive behavior necessitates a better understanding of the degree to which different forms of impulsivity utilize similar neurocircuitry or neurotransmitter systems.

Therefore, this study was designed to test the relationships between scores on several common self-report questionnaires with levels of D1- and D2-tpe receptors in the striatum.
Given the literature showing a negative relationship between dopamine function and impulse control, we predict that dopamine receptors will be negatively related with scores of impulsiveness.

**Materials and Methods**

*Research Participants*

All study procedures were approved by the Institution Review Board of the University of California Los Angeles (UCLA). Thirty-one healthy volunteers (16 female, mean age = 30.68 years, SD = 8.3), recruited from the UCLA Consortium for Neuropsychiatric Phenomics (www.phenomics.ucla.edu), in which they completed extensive neuropsychological testing and underwent MRI scanning as part of larger study of the genetic and environmental bases of variation in psychological and neural phenotypes(Bilder et al., 2009). Each participant provided written informed consent and underwent health screening using the Structured Clinical Interview for DSM-IV, a physical examination, blood and urine screening. Participants were excluded based on the following criteria: Current axis I psychiatric diagnoses other than nicotine dependence; use of psychotropic medications or substances, except marijuana or alcohol (not meeting criteria for dependence); CNS, cardiovascular, pulmonary or systematic disease; HIV seropositivity, hepatic disease and pregnancy. On each day of testing, participants were required to provide a negative urine sample for testing of recent drug use and pregnancy (women only).

*Personality Inventories*

1) Barratt Impulsiveness scale (BIS-11)(Patton et al., 1995)
A widely used and well-validated personality scale, the BIS-11 consists of 30 statements. Subjects are asked to rate each statement on a scale from 1 to 4 of how much the statement describes them. Although there are 3 traditional scoring subscales reported in the literature, several recent structural analyses of the BIS-11 suggest alternative scoring methods for this scale (Reise et al., 2013, Steinberg et al., 2013). Here, we report the total impulsiveness score from the BIS-11, and scores representing cognitive impulsivity and behavioral impulsivity (Reise et al., 2013).

2) Eysenck-Impulsivity (I7) (Eysenck et al., 1985)

Designed to measure impulsivity according to Eysenck’s theory of personality, this a 54-item self-report questionnaire includes subscales describing impulsivity, venturesomeness, and empathy. For the purposes of this analysis, scores from the impulsivity subscale are reported.

3) Multidimensional Personality Questionnaire-Constraint (MPQ) (Patrick et al., 2002)

Scores from the Constraint subscale from the comprehensive MPQ were used as an assessment of impulsivity and are reported here. Notably, high scores on this scale are indicative of high constraint, which correlates to low levels of impulsivity.

4) Dickman Impulsivity Inventory (Dickman, 1990)

This scale distinguishes two types of impulsivity. Dysfunctional impulsivity is the tendency to act with less forethought than most people of equal ability when this tendency is a source of difficulty. Functional impulsivity, in contrast, is the tendency to act with relatively little forethought when such a style is optimal. While these tendencies are not highly correlated, the scores from the dysfunctional impulsivity scale are reported here as they align with other scales.
**PET scanning**

D1-type dopamine receptor availability was assayed using $[^{11}\text{C}]\text{NNC-112}$, a selective ligand with high affinity for D1-type receptors in the striatum (Andersen et al., 1992, Ekelund et al., 2007), in 26 subjects (14 female). Dopamine D2-type receptor availability was assayed using $[^{18}\text{F}]\text{fallypride}$, a radioligand with high affinity for D2-type receptors (Mukherjee et al., 1995) in 27 subjects (14 female). PET scanning was performed on a Philips Gemini Tru Flight PET/CT in 3D mode (Koninklijke Philips Electronics N. V., The Netherlands) (FWHM for brain scanning is 5.0 mm (transverse) × 4.8 mm (axial)). Images with 90 slices were obtained with a 2×2×2-mm³ voxel size and a 128×128 matrix size. A CT-transmission scan was performed to obtain data for measured attenuation correction. After a bolus injection of $[^{11}\text{C}]\text{NNC-112}$ (~15 mCi ± 5%, specific activity $\geq 1 \text{Ci/µmol}$), dynamic emission data were acquired for 90 min as 1-min frames. For $[^{18}\text{F}]\text{fallypride}$ dynamic emission data were acquired after bolus injection (~5 mCi ± 5%, specific activity $\geq 1 \text{Ci/µmol}$) in two scanning blocks of 80-min each, as 1–min frames. To reduce discomfort, participants were allowed a short break between $[^{18}\text{F}]\text{fallypride}$ scanning blocks in which they were instructed to void to reduce radiation exposure to the bladder wall.

Data were reconstructed using Fourier rebinning and filtered back projection, scatter and random corrections were applied.

**PET image processing**

Reconstructed $[^{11}\text{C}]\text{NNC-112}$ PET data (1 min × 90 frames) were averaged into 23 frames, consisting of 1 min × 4 frames, 2 min × 3 frames and 5 min × 16 frames. Reconstructed $[^{18}\text{F}]\text{fallypride}$ PET data (2 blocks; 1 min × 80 frames) were averaged into 16 frames, each consisting of an average of 10-min of dynamic data. PET images were corrected for motion.
(Jenkinson et al., 2002), then co-registered to the corresponding structural MRI (Jenkinson and Smith, 2001).

Time-activity data within each VOI were extracted from motion-corrected, co-registered PET images and imported into PMOD version 3.1 (PMOD Technologies Ltd, Zurich) for kinetic modeling. Time-activity curves were fit using the simplified reference tissue model (SRTM)(Lammertsma and Hume, 1996) and the cerebellum was selected as a reference region, as it has a negligible concentration of D1-type receptors(Hall et al., 1994, Abi-Dargham et al., 2000), 5-hydroxy tryptamine 2A (5-HT2A) receptors(Hall et al., 2000) and D2-type receptors(Vandehey et al., 2010) and displays non-displaceable distribution volume of the radiotracer is similar to that for the tissue of interest: $K1/k2 = K1'/k2'$ (Abi-Dargham et al., 2000). A volume-weighted average of $k2'$, estimated from high-activity regions (caudate and putamen), was computed. The time-activity curves were refit using SRTM2(Wu and Carson, 2002) applying the computed $k2'$ values to all VOIs. Binding potential relative to non-displaceable uptake ($BP_{ND}$) was then calculated by subtracting 1.0 from the product of $R1$ and $k2'/k2a$. Twenty-six and twenty-seven subjects underwent PET scanning with $[^{11}C]NNC-112$ and $[^{18}F]fallypride$, respectively.

**Magnetic resonance image (MRI) acquisition and volumes of interest (VOIs)**

MRI scanning was performed on a Siemens Trio scanner (MPRAGE: repetition time = 1.9 sec, echo time = 2.26 msec, FOV = 250, matrix = 256 × 256, sagittal plane, slice thickness = 1 mm, voxel size = 1 × 1 × 1, 176 slices), and data were processed using the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/index.html Oxford University, Oxford).
Selected striatal VOIs included the whole striatum and 3 functional striatal subdivisions as regions of interest: limbic striatum, associative striatum, and sensory-motor striatum. A VOI for the whole striatum was created by combining anatomically defined VOIs for the caudate, putamen and nucleus accumbens using the FSL software package (FSL-FIRST; Patenaude et al., 2011). Functional subdivisions of the striatum were defined using guidelines described previously (Mawlawi et al., 2001). The cerebellum VOI was drawn manually on the left and right hemispheres in MNI-152 space and then transformed into each subject’s native MRI space.

Data analysis and statistical analysis

Relationships of dopamine receptor BP<sub>ND</sub> with scores from the impulsivity personality scales were tested using partial correlation analysis (SPSS 21; IBM Corp., Armonk, NY) controlling for the effects of age and sex.

In order to assess the effect of both receptor subtypes (D1-type and D2-type BP<sub>ND</sub>) on impulsivity, a generalized linear regression model was performed in SPSS (GLM; SPSS 21; IBM Corp., Armonk, NY). Variables included in the model were D1-type BP<sub>ND</sub>, D2-type BP<sub>ND</sub>, age and sex.
RESULTS

Scores on personality scales of impulsiveness

Scores on personality assessments of impulsiveness were similar to those reported in the literature (Stanford et al., 2009, Reise et al., 2013). The means and standard deviations for each scale are reported in Table 1.

Dopamine receptor $BP_{ND}$

Overall, $BP_{ND}$ values for both receptor subtypes were higher in the dorsal regions of the striatum than in the ventral regions. D1-type $BP_{ND}$ and D2-type $BP_{ND}$ were approximately equal in the associative and sensory-motor striatum, while D2-type $BP_{ND}$ was higher in the sensory-motor striatum than in the associative striatum. D2-type receptor $BP_{ND}$ was higher than that of D1-type $BP_{ND}$ in the midbrain. D1-type receptor $BP_{ND}$ and D2-type $BP_{ND}$ showed no significant correlation in the associative or limbic striatal subdivisions, but in the sensory-motor striatum this correlation was significantly positive ($r = 0.469$, $p = 0.028$). Means and standard deviations are reported in Table 2.

Relationships between impulsivity scales

Overall, the scores from each of the personality assessments showed a high degree of within-subject correlation between scales, although some scales were more correlated than others (Table 3). Scores on the Dickman dysfunctional impulsivity scale and MPQ showed strong correlations with all other examined. In contrast, the Eysenck I7 scale, showed slightly weaker associations with BIS total score and the BIS-11 subscales.
Dopamine receptor $BP_{ND}$ and impulsivity scales (Table 4)

There were no correlations with striatal D1-type $BP_{ND}$ and impulsiveness scores. Midbrain D1-type receptor $BP_{ND}$ was negatively correlated with BIS-11 total score ($r = 0.412$, $p = 0.046$) (Figure 1A) and the BIS-11 behavioral impulsivity subscale score ($r = 0.598$, $p = 0.002$) (Figure 1B). D2-type receptor $BP_{ND}$ in the midbrain and striatum showed no significant correlations with scores on any of the impulsivity scales tested. However a trend-level negative association was found with BIS-11 cognitive impulsivity score and midbrain D2-type receptor $BP_{ND}$ ($r = -0.349$, $p = 0.088$).

**DISCUSSION**

This study extends evidence for a role of dopaminergic signaling in human impulsivity (Buckholtz et al., 2010) by demonstrating the involvement of midbrain D1-type receptors in impulsiveness. In the midbrain, D1-type receptors are selectively localized pre-synaptically on afferent striato-nigral terminals. Activation of D1-type receptors on striato-nigral terminals facilitates the release of GABA acting at $GABA_A$ receptors to increase inhibition of midbrain dopamine neurons (Cameron and Williams, 1993), and decrease dopamine release in the striatum. Dopamine release in the striatum is linked with self-report measures of impulsivity and this relationship is mediated by D2-type receptors in the midbrain (Buckholtz et al., 2010) by

D1-type receptor expression is highest in the substantia nigra (Reyes et al., 2013), with VTA neurons showing lower levels of expression (Reyes et al., 2013). Therefore, the
involvement of dopamine action at D1-type receptors at the midbrain level likely affects striatal signaling.

In combination with the results reported in Chapter 2 (Study 1), the current findings suggest that the behavioral tendencies detected with self-report scales of impulsivity do not capture the same impulsive phenotype as those detected with neurocognitive tasks of inhibition. This is supported in the literature by studies failing to find correlations between self-report and behavioral assessments (Reynolds et al., 2006). Furthermore, the current data imply that dopamine signaling differentially modulates these two types of impulsivity. While the inhibition captured by neurocognitive tasks is likely facilitated by dopaminergic signaling at D1- and D2-type receptors in the striatum (Chapter 2), impulsivity as assessed by personality scales may be governed by dopamine receptor signaling in the midbrain/substantia nigra area. Given the different signaling patterns, dopamine may interact with impulsivity at each of these nodes in the dopaminergic circuitry, thus influencing different aspects of impulsive behavior.

There are several fundamental differences between self-report assessments and neurocognitive tasks. Self-report measures rely on the ability of the subject to recognize their own behavioral predisposition and report it accurately in the context of other individual’s behavior. Varying degrees of self-perception and impression management may affect the accuracy of the data collected, but the “trait-like” qualities may give a more accurate representation of stable behavior patterns. In comparison, neurocognitive task performance is less influenced by self-perception bias and provides a more objective assessment of behavior. However, the aspects of behavior that are captured in neurocognitive tasks tend to be specific behavioral dimensions (inability to inhibit a pre-potent response), and therefore may not translate
well to more general behavioral contexts (resisting drug use). Although studies rarely use both
types of assessments to measure impulsiveness, studies have failed to find correlations between
neurocognitive tasks and personality scales (Reynolds et al., 2006, Meda et al., 2009, Cyders and
Coskunpınar, 2011). Each type of impulsivity assessment captures important information and
future studies of impulsivity would benefit from employing both types of assessments
simultaneously as the emphasis to find biological grounding in human behavior continues.

Although previous studies show that midbrain D2-type receptor availability, and to a
greater extent, amphetamine-induced dopamine release in the striatum are linked to individual
levels of self-reported impulsivity (Buckholtz et al., 2010), no significant relationships were
detected in this sample of healthy controls with D2-type BP\textsubscript{ND}. This apparent discrepancy may
be rooted in the smaller sample size of the current study or the small degree of variation in
impulsivity scores in healthy samples. Certainly, striatal D2-type receptors were correlated with
impulsivity scores in a case-control study of methamphetamine dependence (Lee et al., 2009),
however this relationship was drive by the drug-using subjects, as the correlation in healthy
subjects alone existed at trend-level (Lee et al., 2009). Furthermore, novelty-seeking seems to
play an important role in the correlation between impulsivity and D2-type receptors in the
midbrain (Zald et al., 2008, Buckholtz et al., 2010). However, novelty-seeking characteristics
were not assessed in these subjects and not controlled for as in other studies (Buckholtz et al.,
2010).

This study has limitations. Among them are its correlative design, which cannot inform
on causal relationships between dopamine-receptor subtype signaling and motor response
inhibition, and the relatively small sample size. Another is imperfect selectivity of the radioligands used. $[^{11}\text{C}]\text{NNC-112}$ has approximately 10-fold higher \textit{in vivo} affinity for D1-type over 5HT2A receptors (Slifstein et al., 2007), and pharmacological blocking studies show that \~5\% of the $[^{11}\text{C}]\text{NNC-112}$ signal in the striatum represents 5HT2A binding (Ekelund et al., 2007). Although contamination of the D1-receptor signal in the striatum with 5HT2A binding is minor in the striatum, it should be acknowledged. However, binding of $[^{11}\text{C}]\text{NNC-112}$ to 5HT2A receptors in the midbrain is not negligible, it is likely that the $[^{11}\text{C}]\text{NNC-112}$ signal in this region is a combination of D1-type and 5HT2A receptors binding.

There has been significant progress in improving our understanding of the biological basis of impulsivity and its importance in mental disorders. The current results are important because they expand our understanding of how dopamine signaling is important for impulsiveness by providing evidence that midbrain D1-type receptors are important for this character trait. Although D2-type receptors in this region are linked with impulsiveness, the current data expands our understanding of the role for dopamine signaling through actions at D1-type receptors, suggesting that dopamine signaling is dynamically involved in the expression of impulsive behaviors at multiple levels. While the complete picture of the biological underpinnings of impulsivity is not yet clear, increasing this knowledge will improve our definitions of impulsivity and how it relates to treatment and prevention of drug use disorders.
Figures

Figure 3.1: Scatter plots of the correlations between D1-type BP<sub>ND</sub> in the midbrain with self-reported measures of impulsiveness on the BIS-11. (A) Total score BIS-11 and (B) Behavioral impulsivity subscale, BIS-11.
Table 3.1: Means and standard deviations of impulsiveness scores from personality scales

<table>
<thead>
<tr>
<th></th>
<th>mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIS-11 (total)</strong></td>
<td>56.0 (9.5)</td>
</tr>
<tr>
<td>Cognitive</td>
<td>5.7 (1.6)</td>
</tr>
<tr>
<td>Behavioral</td>
<td>4.9 (1.5)</td>
</tr>
<tr>
<td><strong>Dickman: dysfunctional</strong></td>
<td>1.5 (2.3)</td>
</tr>
<tr>
<td><strong>MPQ</strong></td>
<td>18.3 (5.4)</td>
</tr>
<tr>
<td><strong>Eyesenck I7</strong></td>
<td>6.0 (3.2)</td>
</tr>
</tbody>
</table>
Table 3.2: Correlations between D1-type and D2-type BP\textsubscript{ND} in the striatum and midbrain.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>D1-type BP\textsubscript{ND} mean (SD)</th>
<th>D2-type BP\textsubscript{ND} mean (SD)</th>
<th>Correlation r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Striatum</td>
<td>1.98 (0.19)</td>
<td>29.59 (4.68)</td>
<td>0.310</td>
</tr>
<tr>
<td>Limbic Striatum</td>
<td>1.82 (0.18)</td>
<td>26.38 (4.26)</td>
<td>0.193</td>
</tr>
<tr>
<td>Associative Striatum</td>
<td>2.02 (0.23)</td>
<td>29.29 (4.55)</td>
<td>0.259</td>
</tr>
<tr>
<td>Sensory-Motor Striatum</td>
<td>2.01 (0.21)</td>
<td>31.87 (5.77)</td>
<td>0.469*</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.238 (0.04)</td>
<td>2.45 (0.36)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

* p = 0.028
n=26 (14F)  n=27 (14F)  n=22 (12F)
Table 3.3: Correlations between scores on personality scales

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BIS-11: cognitive</td>
<td>0.738**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIS-11: behavioral</td>
<td>0.667**</td>
<td>0.316</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dickman: dysfunctional</td>
<td>0.676**</td>
<td>0.579**</td>
<td>0.537**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPQ: constraint</td>
<td>-0.696**</td>
<td>-0.533**</td>
<td>-0.548**</td>
<td>-0.832**</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyesenck: I7</td>
<td>0.430*</td>
<td>0.302</td>
<td>0.293</td>
<td>0.751**</td>
<td>-0.502**</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>0.099</td>
<td>0.111</td>
<td>&lt; 0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Table 3.4: Correlations of D1-type and D2-type BP<sub>ND</sub> in the striatum and midbrain with scores from personality scales of impulsiveness.

<table>
<thead>
<tr>
<th></th>
<th>D1-type BP&lt;sub&gt;ND&lt;/sub&gt;</th>
<th></th>
<th>D2-type BP&lt;sub&gt;ND&lt;/sub&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Striatum</td>
<td>Midbrain</td>
<td>Striatum</td>
<td>Midbrain</td>
</tr>
<tr>
<td>BIS-11: total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>-0.08</td>
<td>-0.412</td>
<td>-0.198</td>
<td>-0.213</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.711</td>
<td>0.046</td>
<td>0.344</td>
<td>0.306</td>
</tr>
<tr>
<td>BIS-11: cognitive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.223</td>
<td>0.034</td>
<td>-0.314</td>
<td>-0.349</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.294</td>
<td>0.875</td>
<td>0.126</td>
<td>0.088</td>
</tr>
<tr>
<td>BIS-11: behavioral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>-0.193</td>
<td>-0.598</td>
<td>-0.042</td>
<td>-0.024</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.367</td>
<td>0.002</td>
<td>0.842</td>
<td>0.908</td>
</tr>
<tr>
<td>Dickman: dysfunctional</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.093</td>
<td>-0.153</td>
<td>-0.263</td>
<td>-0.303</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.666</td>
<td>0.477</td>
<td>0.205</td>
<td>0.141</td>
</tr>
<tr>
<td>MPQ: constraint</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>-0.265</td>
<td>0.244</td>
<td>0.198</td>
<td>0.221</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.211</td>
<td>0.251</td>
<td>0.343</td>
<td>0.289</td>
</tr>
<tr>
<td>Eyesenck: I7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>-0.025</td>
<td>0.017</td>
<td>-0.247</td>
<td>-0.199</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.907</td>
<td>0.937</td>
<td>0.233</td>
<td>0.341</td>
</tr>
</tbody>
</table>
References for Chapter 3


dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. J Neurosci 29:14734-14740.


CHAPTER 4: EFFECT OF EXERCISE TRAINING ON STRIATAL DOPAMINE D2-TYPE RECEPTORS IN METHAMPHETAMINE USERS DURING BEHAVIORAL TREATMENT

THIS CHAPTER IS A VERSION OF A MANUSCRIPT THAT HAS BEEN SUBMITTED FOR REVIEW:

SUMMARY (ABSTRACT)

Methamphetamine use disorder is associated with striatal dopaminergic deficits, which have been linked to poor treatment outcomes, identifying these deficits as an important therapeutic target. Exercise attenuates methamphetamine-induced neurochemical damage in the rat brain, and a preliminary observation suggests that exercise increases striatal D2-type receptor availability (measured as non-displaceable binding potential, BPND) in patients with Parkinson’s disease. The goal of this study was to evaluate whether adding an exercise-training program to an inpatient behavioral intervention for methamphetamine use disorder reverses deficits in striatal D2-type receptors.

Participants were adult men and women who met DSM-IV criteria for methamphetamine dependence and were enrolled in a residential facility, where they maintained abstinence from illicit drugs of abuse and received behavioral therapy for their addiction. They were randomized to a group that received supervised exercise training (n=10) or one that received equal-time health-education training (n=9), 3 days/week for 8 weeks. They came to an academic research center for positron emission tomography (PET) using [18F]fallypride to determine the effects of the 8-week interventions on striatal D2-type receptor BPND.

Repeated measures ANOVA showed a significant effect of the treatment group X time interaction on striatal D2-type BPND, which reflected in an increase of striatal D2-type BPND in the exercise group, but not in the education group after 8 weeks.

These findings suggest that structured exercise training can ameliorate striatal D2-type receptor deficits in methamphetamine users, and warrants further evaluation as an adjunctive treatment for stimulant dependence.
INTRODUCTION

Deficits in markers of the striatal dopaminergic system are hallmark features of substance-use disorders (Volkow et al., 2009, Broft and Martinez, 2012), and likely reflect both genetic predisposition and molecular adaptions to repeated drug exposure (Everitt and Robbins, 2005, Nader and Czoty, 2005, Groman et al., 2012, Groman and Jentsch, 2013, Volkow and Baler, 2014). Human neuroimaging studies have shown that chronic stimulant users display deficits in striatal dopamine receptors (Volkow et al., 1990, Lee et al., 2009), dopamine transporters (McCann et al., 1998, Volkow et al., 2001c), vesicular monoamine transporters (Johanson et al., 2006, Narendran et al., 2012), dopamine synthesis (Baxter et al., 1988), endogenous striatal dopamine levels, (Martinez et al., 2009), and presynaptic dopamine release (Volkow et al., 1997, Wang et al., 2012, Volkow et al., 2014).

Other studies have shown a gradual recovery of dopaminergic markers with sustained drug abstinence, with striatal dopamine-transporter availability being 20% greater in subjects who were abstinent 12-17 months compared with those who were abstinent for only 6 months (Volkow et al., 2001b). The rate of recovery of dopamine transporter availability varies widely between individuals, and some deficits persist long after cessation of drug use (Sekine et al., 2001, McCann et al., 2008). In non-human primates, methamphetamine-induced decreases in striatal D2-type dopamine receptors persist for over 7 weeks (Groman et al., 2012), while similar cocaine-induced deficits persist for up to 1 year (Nader et al., 2006). There are no published reports of recovery of D2-type receptors with drug abstinence in human subjects.

PET imaging studies show that low levels of D2-type receptors are associated with drug use and associated behaviors (Parvaz et al., 2011). In recently abstinent methamphetamine-
dependent individuals (4-7 days), D2-type receptor availability is negatively associated with self-reported impulsivity (Lee et al., 2009), discounting of delayed rewards (Ballard et al., 2015), and caloric intake, suggesting that food is used as a substitute reinforcer in a reward-deficiency syndrome (Zorick et al., 2012). Moreover, D2-type receptor availability and even more so, function of the striatal element, as inferred from dopamine release, was positively associated with treatment outcomes for stimulant users (Martinez et al., 2011, Wang et al., 2012).

Individual variation in striatal dopamine D2-type BP\textsubscript{ND} has been linked to neural activity and associated cognitive function in domains such as risky-decision making (Kohno et al., 2015) and response inhibition (Monterosso et al., 2005, Ghahremani et al., 2012), in which stimulant abusers show deficits. Therefore, increasing signaling through D2-type receptors represents a potentially important therapeutic target in the care of individuals with addiction (Wang et al., 2004).

In rodents, exercise augmented striatal dopamine concentrations, dopamine receptor binding and tyrosine hydroxylase mRNA (Gilliam et al., 1984, MacRae et al., 1987, Petzinger et al., 2007, Foley and Fleshner, 2008, Greenwood et al., 2011). Other studies have shown that wheel running attenuates methamphetamine-induced damage to serotonergic and dopaminergic terminals (O'Dell et al., 2012, O'Dell S and Marshall, 2014) (Marshall and O'Dell, 2012), as well as D2-type dopamine receptor binding in a model of Parkinson’s disease (Vuckovic et al., 2010). Moreover, in a pilot study of four patients with early-stage Parkinson’s disease, striatal D2-type BP\textsubscript{ND} was increased in the two patients who engaged in treadmill exercise but not in the two who did not (Fisher et al., 2013).
Although it has been suggested that exercise can boost dopamine function during early abstinence from drug use (Smith and Lynch, 2011, Lynch et al., 2013), the effects of exercise on the dopamine system have not been studied in humans with addictions. In the present study, therefore, PET was used with $[^{18}\text{F}]$fallypride as a radioligand for dopamine D2-type receptors (Mukherjee et al., 1995) to examine the effect of an 8-week exercise-training program on striatal D2-type receptor $\text{BP}_{\text{ND}}$ in individuals undergoing behavioral treatment for methamphetamine addiction in a residential facility.

**METHODS**

*Study design*

Subjects recruited into this brain-imaging study were concurrently participating in a larger randomized, controlled trial of exercise compared to health education for methamphetamine dependence ($n=135$) designed to test the impact of an 8-week aerobic exercise regimen versus an 8-week health education program on MA dependent individuals who are receiving treatment at a residential treatment facility (Mooney et al., 2014). Those who met entry criteria and expressed interest in continuing were invited to participate in this sub-study, involving brain-scanning procedures. All participants continued with the regular schedule of treatment activities at the facility, including group and individual therapy and 12-step meetings. Screening to determine eligibility included a medical history, physical examination, clinical laboratory tests and a 12-lead resting electrocardiogram (ECG). All study-related procedures were approved by the Institution Review Boards of the University of California Los Angeles (UCLA) and the Greater Los Angeles Veterans Affairs Health Care System. Participants were recruited to this study within 72 h of admission to a residential facility in Southern California (CRI-Help Inc., George T. Pfleger Rehabilitation Center, North Hollywood, CA), which they
entered for addiction treatment. Each participant was fully informed of the benefits and risks of the study and provided written consent to participate.

Participants

Participants were required to be 18-55 years of age, to reside at the treatment center, and to meet DSM-IV-TR criteria for methamphetamine dependence as determined via the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Individuals were excluded if they met criteria for any other axis 1 disorders or dependence on any other drugs of abuse besides nicotine and marijuana. All participants completed a comprehensive medical examination including a urine screen for recent drug use and pregnancy (females only). Exclusionary criteria included any musculoskeletal conditions and unstable cardiovascular, pulmonary, metabolic, or other disorders that would preclude participation in exercise training. Participants with conditions that could either interfere with the acquisition of the neuroimaging data or for whom the neuroimaging procedures would pose a potential risk were excluded (i.e. implanted metal objects in the body, claustrophobia and use of medications known to interact with D2-type receptors).

Baseline assessment

All participants completed a baseline maximal incremental exercise test using a symptom-limited incremental treadmill protocol described previously (Dolezal et al., 2013, Dolezal et al., 2014). Briefly, participants were asked to walk on a treadmill with gradually increasing speed and grade as long as they could. Individual performance on the baseline exercise test was used to set parameters for the training intensity of subsequent exercise sessions for each of the exercise-group participants.
**Exercise training group (EX)**

Each participant randomized to the EX group participated in 1-hour individualized exercise sessions 3 days/week for 8 weeks, under the supervision of an experienced exercise trainer, in the gym located within the treatment facility. Participants walked and/or jogged on a treadmill for 30 minutes at an intensity determined from the individual’s baseline exercise performance. Participants also completed a circuit-type resistance-training program, using weight machines and/or dumbbell free weights, which included all the major muscle groups of the upper and lower body (Dolezal et al., 2013, Dolezal et al., 2014, Mooney et al., 2014).

**Education control group (ED)**

Each participant randomized to the ED group participated in small-group (< 5 participants) health-education sessions for approximately 1 hour, 3 times/week for 8 weeks. A trained counselor conducted sessions addressing a variety of health, wellness, and lifestyle topics such as healthy eating, dental care, acupressure, and cancer screening (Mooney et al., 2014). There was no guidance or encouragement to engage in exercise training although participants in this group did have access to the in-house gym, like all other residents, if they wished.

**PET scanning**

Dopamine D2-type receptor availability (D2-type BP$_{ND}$) was determined before and after the 8-week intervention in both EX and ED groups. D2-type BP$_{ND}$ was determined using $[^{18}\text{F}]$fallypride, a radioligand with high affinity for D2-type receptors (Mukherjee et al., 1995). PET scanning was performed on a Philips Gemini Tru Flight PET/CT in 3D mode (FWHM 5.0 mm $\times$ 4.8 mm, Philips Electronics NV, Netherlands). A CT-transmission scan was performed to
obtain data for measured attenuation correction. After bolus injection of $[^{18}\text{F}]$fallypride (~5 mCi ± 5%, specific activity ≥ 1 Ci/µmol), dynamic emission data were acquired in two scanning blocks of 80 min each, as 1-min frames. To reduce discomfort and radiation exposure to the bladder wall, participants were allowed a short break between scanning blocks to leave the scanning bed and to void urine. Data were reconstructed using the 3D row action maximum likelihood algorithm (3D-RAMLA). Scatter and random corrections were applied.

**MRI scanning and volumes of interest (VOIs)**

MRI scanning was performed on a Siemens Trio (MPRAGE: repetition time = 1.9 sec, echo time = 2.26 msec, voxel size = $1 \times 1 \times 1$ mm$^3$, 176 slices), and processed using the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/index.html, Oxford University). Selected volumes of interest (VOIs) included the whole striatum and its functional subdivisions: limbic striatum, associative striatum, and sensory-motor striatum. Extrastriatal VOIs for the hippocampus, amygdala and thalamus were defined using the FSL software package (Patenaude et al., 2011). A whole-striatal VOI was created by combining VOIs of the caudate, putamen and nucleus accumbens (Patenaude et al., 2011). The functional subdivisions of the striatum (Mawlawi et al., 2001) and midbrain (Zald et al., 2010) were defined using published guidelines. The cerebellum was selected as an appropriate reference region (Hall et al., 1994, Ishibashi et al., 2013). A VOI was drawn manually in MNI-152 space as a bilateral region encompassing the both hemispheres, while avoiding the vermis. The standard-space VOI was then transformed to each subject’s native MRI (Ishibashi et al., 2013).

**PET image processing**

Reconstructed $[^{18}\text{F}]$fallypride PET data (2 blocks; 1-min × 80-frames) were combined into 16 frames, each consisting of the average of 10-min. PET images were motion-corrected...
(Jenkinson et al., 2002) then co-registered to the corresponding MRI (Jenkinson and Smith, 2001). VOI-based time/activity data were extracted for kinetic modeling using PMOD (PMOD 3.1, Zurich). Time/activity curves were fit using the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996). A volume-weighted average of k2', estimated from high-activity regions (caudate and putamen), was computed. Time/activity curves were then refit using SRTM2 (Wu and Carson, 2002) applying the computed k2' values to all VOIs. Binding potential referred to non-displaceable uptake (BPND) was calculated by subtracting 1.0 from the product of R1 and k2'/k2a.

Statistical analysis

VOIs of the whole striatum and three functional striatal subdivisions were selected a priori due to the high degree of dopaminergic innervation in these regions and existing literature identifying dopaminergic transmission in these regions to be important in the neurobiology of methamphetamine dependence (Lee et al., 2009, Volkow et al., 2009, Wang et al., 2012). Exploratory investigations involved D2-type BPND measurements from other subcortical regions, specifically the amygdala, midbrain, thalamus and hippocampus.

Group differences in baseline BPND (ED vs EX) were evaluated using un-paired t-tests conducted in SPSS (IBM SPSS 22.0; Chicago, IL). Comparisons were made for BPND measurements from the whole striatum VOI. Group differences in BPND for functional striatal subdivisions were compared using post-hoc t-tests if a significant difference was first identified using the whole striatum VOI. Group differences in demographic variables (ED vs EX) were compared using unpaired t-tests using SPSS (IBM SPSS 22.0; Chicago, IL).
Changes in whole striatal \( \text{BP}_{\text{ND}} \) between baseline and post-intervention measurements were examined using a repeated-measures analysis of variance (ANOVA) in SPSS (IBM SPSS 22.0; Chicago, IL). Taking whole striatal \( \text{BP}_{\text{ND}} \) as the dependent variable, the main effects of time (baseline vs post-intervention) and group (ED vs EX) were included in the model, the interaction effect (time x group) was also included. Post-hoc paired t-tests were used to test the changes in striatal \( \text{BP}_{\text{ND}} \) within each treatment group.

**RESULTS**

**Participants**

Thirty-one subjects (mean age 29.8 +/- 5.9 years) were recruited to participate in the brain-imaging study; 16 were randomized to the EX group (10 men, 6 women), and 15 to the ED group (7 men, 8 women). All underwent PET scanning at baseline, but 12 subjects did not complete the protocol: 6 from the EX group and 6 from the ED group. Eleven of the non-completers chose to leave the treatment facility, and one participant was withdrawn for medical reasons. Nineteen subjects underwent post-intervention PET scanning after completing 8 weeks of exercise-training (n=10) or the health-education program (n=9).

**Demographics**

Participants in the EX and ED groups did not differ on any of the demographic variables examined (Table 1). Most were smokers (n = 29) and had completed an average of 11.8 years of education. On average, they reported using methamphetamine for approximately 8.6 years, estimating that they had used methamphetamine on 20 of the 30 days preceding admission to the treatment facility. The subjects who withdrew from the study did not differ from those who completed the protocol on any of the baseline demographic variables, except for years of
methamphetamine use. Those who withdrew (n=12) had more years of methamphetamine use than those who completed (n=19) the protocol: Mean (SD) years 14.7 (6.0) versus 8.6 (4.3); p = 0.006.

Participants continued treatment as usual at the residential facility, and had similar clinical activities, social responsibilities and daily meal options. Details of the study design and procedures have been reported previously (Mooney et al, 2014). On average, subjects completed approximately 22 of 24 possible sessions, with no group difference in the number of sessions (mean (SD) completed: EX 22.2 (1.8) versus ED 22.4 (1.9) sessions. Body-weight increased on average by 6.7% in the ED group after 8-weeks (p= 0.020), while mean body-weight did not change in the EX group.

Effects of EX and ED interventions on striatal D2-type dopamine receptor BP_{ND}

Baseline striatal D2-type BP_{ND} was approximately equal between the two treatment groups in all striatal regions (Table 2). Results from the repeated-measures ANOVA showed a main effect of time point on D2-type BP_{ND} (F_{1,17} = 10.591, p = 0.005), and a significant treatment group x time point interaction (F_{1,17} = 4.135, p = 0.058), indicating a specific effect of exercise on striatal BP_{ND}. Post-hoc paired t-tests show a significant increase in the whole striatum D2-type BP_{ND} in the EX group, (t = 3.917, p = 0.004) but not in the ED group (t=0.848, p=0.421). All functional striatal subdivisions showed similar increases in the EX group by paired t-tests: limbic: t = 3.04, p = 0.014, associative: t = 3.51, p = 0.007 and sensory-motor: t = 3.691, p = 0.005.
On average, participants in the EX group displayed a 14.5% increase in D2-type BPND in the whole striatum whereas those in the ED group displayed an average increase of 3.9%. EX group participants showed, on average, increases of 15.8% and 16.3% in the associative and sensory-motor regions, respectively, and 8.1% in the limbic striatum. ED group participants showed average increases of 5.1%, 4.3%, and 4.0% in the limbic, associative and sensory-motor regions of striatum, respectively.

Notably, at baseline both groups displayed lower D2-type BPND than that of a separate group of healthy control subjects examined using the same imaging procedures (Robertson et al., 2015 in press). However, post-intervention BPND in the exercise group, but not the education group, was comparable to BPND values of healthy subjects. For reference, BPND values for healthy comparison subjects [n=28 14 men/14 women; (mean (SD): Whole Striatum 29.72 (4.64); Limbic 26.58 (4.32), Associative 29.40 (4.50); Sensory motor 32.00 (5.70)] (Robertson et al., 2015 in press).

Effects in extrastriatal regions

Exploratory investigations of D2-type BPND measurements in the amygdala, hippocampus, thalamus and midbrain showed no group differences in BPND at baseline nor post-intervention (see Table 3) BPND mean (sd). EX: amygdala: 2.68 (0.35), hippocampus: 1.01 (0.23); thalamus 2.34 (0.32); midbrain: 2.44 (0.28). ED: amygdala: 2.67 (0.41), hippocampus: 1.05 (0.36), thalamus: 2.44 (0.49), midbrain 2.43 (0.21). No group differences in post-intervention BPND were found (BPND mean (sd). EX: amygdala: 2.76 (0.31), hippocampus: 1.06 (0.21), thalamus: 2.44 (0.26), midbrain: 2.45 (0.18). ED: amygdala 2.71 (0.38), hippocampus: 1.09 (0.24), thalamus: 2.53 (0.44), midbrain 2.30 (0.09). There were no significant changes from
baseline at the post-intervention PET scan, except for thalamus $BP_{ND}$, which showed a trend level increase of 4.1 % in the ED group ($t = 2.18, p = 0.060$).

**DISCUSSION**

The present findings add to a preliminary report of an effect of exercise on striatal dopamine receptors in humans (Fisher et al., 2013), and on methamphetamine neurotoxicity in rodents (Marshall and O'Dell, 2012, O'Dell et al., 2012, O'Dell S and Marshall, 2014) by providing evidence that exercise increases striatal D2-type $BP_{ND}$ in methamphetamine users. Although 8-weeks of drug abstinence in addition to behavioral treatment did not significantly change striatal D2-type dopamine receptor availability, addition of an exercise-training program produced significant increases. These findings demonstrate that methamphetamine-associated damages to the D2-type system due to chronic drug use are reversible in human subjects, and that recovery of the dopamine system after chronic drug use can be facilitated with exercise training.

A deficit in striatal D2-type dopamine receptor binding is a common feature across substance-use disorders (Volkow et al., 2009, Bonci, 2013). In stimulant dependence, this deficit is linked to treatment success rates (Martinez et al., 2011, Wang et al., 2012) and measures of impulsivity (Lee et al., 2009) and decision-making (Ballard et al., 2015). Although these observations suggest that augmenting signaling at the D2-type receptor system may be an effective therapeutic target in substance-use disorders (Diana, 2011), dopamine agonist therapy has shown limited success in improving treatment outcomes for stimulant dependence (Rawson
et al., 2002, Ling et al., 2006, Heinzerling et al., 2010, Brensilver et al., 2012). Given the intricate bi-phasic signaling motif of the dopamine system via D1-type and D2-type receptor-mediated pathways (Dreyer et al., 2010, Gerfen and Surmeier, 2011), it is possible that indirect dopamine agonists would have a larger effect on D1-type over D2-type receptor signaling, thus disrupting the balance between striatal dopaminergic pathways. This effect would likely be further compounded in patients with D2-type receptor deficits, such as those seen in drug addictions (Broft and Martinez, 2012). Moreover, pharmacological treatment with non-selective D2-type receptor agonists may have untoward effects by augmenting D3 versus D2 receptor signaling. Notably, stimulant users show greater D3 receptor availability in the limbic striatum compared with healthy control subjects (Payer, 2013). Therefore, non-pharmacologic approaches, such as structured exercise training, that augment dopaminergic signaling in physiologically relevant ways may be useful in treating those with substance use disorders.

Several lines of evidence suggest that high levels of striatal D2-type dopamine receptors may be protective for an individual against drug addiction. For example, unaffected family members of those with alcohol-use disorders display higher D2-type receptor availability than affected family members, and higher D2-type receptor availability than non-related healthy-control subjects, supporting the hypothesis that high levels of D2-type receptors may protect against alcoholism (Volkow et al., 2006). PET studies of stimulant use in humans show an inverse relationship between D2-type receptor availability and positive subjective response to intravenous methylphenidate administration. Individuals with high D2-type receptor availability report more unpleasant subjective effects, suggesting that they may be less vulnerable to stimulant abuse (Volkow et al., 1999, Volkow et al., 2002). Moreover, striatal D2-type receptor availability in drug-naïve rhesus monkeys is negatively correlated with the amount of drug taken.
by animals when trained to self-administer cocaine (Nader et al., 2006). Finally, rats exhibit decreased alcohol self-administration after subjected to an adenoviral-mediated increase in striatal D2 receptor expression (Thanos et al., 2001).

In animal models, exercise produces the most pronounced effects on striatal dopaminergic markers in the dopamine-depleted, compared to non-depleted, striatum. In the MPTP animal model of Parkinson’s disease, six weeks of treadmill exercise produces larger increases in D2-type BP_{ND} in animals treated with MPTP than those treated with saline (Petzinger et al., 2007, Vuckovic et al., 2010). In rodent models of methamphetamine-induced dopamine depletion, exercise increases striatal levels of dopamine transporter and tyrosine hydroxylase in the methamphetamine-treated animals, with only minimal effects in saline-treated animals (O'Dell et al., 2012). Analogously, exercise increased D2-type BP_{ND} by ~80% in two patients with early-stage Parkinson’s disease, while only increasing D2-type BP_{ND} by ~9% in a healthy control subject (Fisher et al., 2013). Although healthy control subjects were not examined in this investigation, exercise-induced increases in D2-type receptors observed here were confined only to regions that show dopaminergic deficits in stimulant users (Volkow et al., 2001a, Lee et al., 2009).

Hypofunction of the striatal dopaminergic system occurs during abstinence from stimulant use and is associated with anhedonia, negative affect, and drug craving (Koob and Volkow, 2010). Methamphetamine-dependent individuals report symptoms of depression, anxiety and drug craving (London et al., 2004, Zorick et al., 2010). Results from the larger clinical trial (Mooney et al., 2014) in which all subjects included in this study were enrolled, showed significant effects of the exercise regimen to improve symptoms of depression and
anxiety (Rawson, 2015, submitted for publication). The neuroimaging findings presented here suggest that exercise-induced increases in D2-type BP\textsubscript{ND} may contribute to these behavioral effects, but direct examination of this relationship in the small neuroimaging sub-sample failed to detect any significant associations between improvements in ratings of affect and changes D2-type dopamine receptor BP\textsubscript{ND}. Additional research is needed to identify the neurobiological mechanisms that underlie exercise-induced amelioration of negative affect in stimulant users.

While the molecular effects of aerobic exercise are incompletely understood, exercise increases levels of neurotrophic factors, and enhances neurogenesis, immune function and neuroplasticity (Cotman and Berchtold, 2002). Mechanisms by which exercise induces up-regulation of D2-type receptors have been investigated in animal models of striatal dopaminergic injury, modeling Parkinson’s disease physiology (Toy et al., 2014). In rodents, wheel running after stimulant exposure produces significant changes in gene transcription factors capable of modulating dopaminergic neurotransmission in the mesolimbic pathways (Greenwood et al., 2011, Zlebnik et al., 2014), and wheel running attenuates MPTP-induced damage to dopaminergic cells in wild-type mice, but not in BDNF (+/-) knockdown mice (Gerecke et al., 2012). In a different model of Parkinson’s disease, wheel running prior to an inflammation-induced injury using lipopolysaccharide (LPS, 1 mg/kg), completely prevented loss of dopaminergic neurons, but blocking BDNF signaling using a TrkB antagonist abolished this effect (Wu et al., 2011). Similarly, BDNF receptor antagonists blocked the effects of treadmill running against damage to dopaminergic neurons, as indicated by preserved levels of tyrosine hydroxylase activity, in a rat model of Parkinson's disease using striatal injection of 6-hydroxydopamine (Real et al., 2013).
Some limitations of this study warrant mention. The first relates to the relatively small size of the sample and the fact that findings may not generalize to all methamphetamine users or to those who are not exposed to exercise in a treatment setting. Generalizability of the findings may be compromised by exclusion of individuals with Axis I disorders, other than methamphetamine dependence because such psychiatric co-morbidity is common among methamphetamine users. Although cigarette use was controlled during scan days, subjects continued to smoke throughout the study, and associations of smoking with D2-type receptor BPND have been reported. Notably smokers, particularly men, have lower D2-type BPND compared to nonsmokers (Fehr et al., 2008, Brown et al., 2012). It is possible that the effects of exercise on D2-type receptors in methamphetamine users may differ according to smoking behavior.

Another limitation of the study is the imperfect selectivity of [18F]fallypride, which has nearly equal affinity for D2 and D3 dopamine receptors in vivo and cannot therefore distinguish between them (Slifstein et al., 2004). Thus, BPND measurements primarily reflect a combination of signals from D2 and D3 receptors. [18F]fallypride also binds to both isoforms of the D2 receptor (D2S and D2L), and therefore does not distinguish between pre- and post-synaptic D2 receptors. Furthermore, the cellular location and integrity of D2-type receptors bound to [18F]fallypride is uncertain. Presumably, membrane-bound receptors constitute the majority of the [18F]fallypride signal, however the degree to which these receptors are functionally active or located on the cell surface is unknown in these studies. It is possible that [18F]fallypride may show differential binding to functionally inactive or intracellular D2-type receptors. Conversely, it is possible that [18F]fallypride could signal reflect receptor binding from a pool of behaviorally relevant D2-type receptors located at the cell surface and a pool of irrelevant D2-type receptors.
that are inactive or intracellular. It is possible that the results from this study reflect exercise-induced changes in the expression of inactive or intracellular D2-type receptors, producing an increase in $[^{18}\text{F}]$fallypride $\text{BP}_{\text{ND}}$ that does not reflect changes in behaviorally relevant D2-type receptors. Future studies of the relationship between exercise-induced changes in behavior and cognition with exercise-induced changes in the brain are needed to determine the degree to which exercise produces functionally relevant changes in D2-type receptors.

While the focus of this investigation is the dopaminergic system, exercise has many effects on other many other neurotransmitter systems, including serotonin, glutamate and opioid systems (Greenwood et al., 2011). In addition, the effects of exercise on physiological systems outside of the brain are numerous, including cardio-vascular, immune and endocrine systems (Whelton et al., 2002, Penedo and Dahn, 2005, Hillman et al., 2008). Earlier findings from a different subsample of the same clinical trial of exercise and methamphetamine dependence (Mooney et al., 2014) showed that exercise training improved heart rate variability, an index of autonomic nervous system that is abnormal in methamphetamine users (Dolezal et al., 2014). Investigations to assess the degree to which exercise-induced changes in physiological measures are related to changes in neurobiological indices would prove clinically useful as a method to assess disease severity (Devos et al., 2003), or to monitor response to treatment and recovery progression.

The current findings contribute to a growing literature identifying the therapeutic benefits of aerobic exercise in health and disease (Cotman and Berchtold, 2002, Vina et al., 2012), and also in the treatment of substance abuse (Lynch et al., 2013). Exercise is gaining attention as a complement to traditional pharmacological and psychotherapeutic treatments. Therefore,
investigating the effects of exercise in patients with neuropsychiatric disorders featuring
dopaminergic dysfunction; such as Parkinson’s disease (Dagher and Robbins, 2009), drug
addictions (Bonci, 2013), and attention deficit disorder (ADHD) (Volkow et al., 2011), is of
increasing clinical relevance. Understanding the molecular mechanisms by which exercise
affects dopaminergic signaling in patients with stimulant-use disorders may produce new clinical
approaches to enhance treatment outcomes for addictions and other related neuropsychiatric
disorders.
**Figures**

Figure 4.1: Bar graph depicting change in mean striatal D2-type $\text{BP}_{ND}$ after completion of an 8-week Education (A) and Exercise (B) program. * $p < 0.01$ using paired t-tests to compare baseline to post-intervention $\text{BP}_{ND}$ values. Errors bars represent 1 +/- SEM.
Table 4.1: Characteristics of Participants subjects in the Exercise and Education groups.

<table>
<thead>
<tr>
<th></th>
<th>Education</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (men/women)</td>
<td>9 (4/5)</td>
<td>10 (7/3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.4 (7.1)</td>
<td>29.0 (3.83)</td>
</tr>
<tr>
<td>Number of Smokers</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Years of Education</td>
<td>11.33 (3.0)</td>
<td>11.78 (1.2)</td>
</tr>
<tr>
<td>Years of Methamphetamine Use</td>
<td>8.8 (4.7)</td>
<td>8.5 (4.1)</td>
</tr>
<tr>
<td>Days of Methamphetamine Use in last 30 days</td>
<td>25 (8.6)</td>
<td>19.5 (10.3)</td>
</tr>
<tr>
<td>Sessions Completed (avg)</td>
<td>22.4</td>
<td>22.2</td>
</tr>
</tbody>
</table>
Table 4.2: Striatal D2-type BP$_{ND}$ at Baseline and Post-intervention. Values represent means (SD). * $p < 0.01$ paired t-tests to compare baseline BP$_{ND}$ to post-intervention BP$_{ND}$.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Striatum</td>
<td>26.44 (5.31)</td>
<td>27.27 (5.26)</td>
</tr>
<tr>
<td>Limbic</td>
<td>23.50 (4.26)</td>
<td>24.64 (4.77)</td>
</tr>
<tr>
<td>Associative</td>
<td>25.66 (5.24)</td>
<td>26.37 (5.76)</td>
</tr>
<tr>
<td>Sensory motor</td>
<td>29.57 (6.59)</td>
<td>30.31 (5.61)</td>
</tr>
<tr>
<td><strong>Exercise Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Striatum</td>
<td>25.80 (3.67)</td>
<td>29.38 (3.90)*</td>
</tr>
<tr>
<td>Limbic</td>
<td>24.12 (2.94)</td>
<td>25.96 (2.84)*</td>
</tr>
<tr>
<td>Associative</td>
<td>25.31 (3.99)</td>
<td>29.09 (4.25)*</td>
</tr>
<tr>
<td>Sensory motor</td>
<td>27.57 (3.83)</td>
<td>31.96 (4.94)*</td>
</tr>
</tbody>
</table>

Values represent means (SD). * $p < 0.01$ paired t-tests to compare baseline BP$_{ND}$ to post-intervention BP$_{ND}$. 
Table 4.3: Extrastriatal D2-type BP_{ND} at Baseline and Post-intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education Group</strong></td>
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<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>2.67 (0.41)</td>
<td>2.71 (0.38)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.05 (0.36)</td>
<td>1.09 (0.24)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.44 (0.49)</td>
<td>2.53 (0.44)</td>
</tr>
<tr>
<td>Midbrain</td>
<td>2.43 (0.21)</td>
<td>2.30 (0.09)</td>
</tr>
<tr>
<td><strong>Exercise Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>2.68 (0.35)</td>
<td>2.76 (0.31)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.01 (0.23)</td>
<td>1.06 (0.21)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.34 (0.32)</td>
<td>2.44 (0.26)</td>
</tr>
<tr>
<td>Midbrain</td>
<td>2.44 (0.28)</td>
<td>2.45 (0.18)</td>
</tr>
</tbody>
</table>
References for Chapter 4


nicotine dependence similar to that seen with other drugs of abuse. Am J Psychiatry 165:507-514.


CHAPTER 5: SUMMARY, DISCUSSION AND FUTURE DIRECTIONS
SUMMARY OF FINDINGS

These studies used positron emission tomography techniques to investigate how individual differences in markers of striatal dopamine receptors are related to different forms of inhibitory control and impulsivity, and to examine how aerobic exercise effects markers of striatal dopamine receptors in methamphetamine use disorder.

Study 1 (Chapter 2) showed that individual variation in both D1- and D2-type receptor BP$_{ND}$ are associated with performance on a motor response inhibition task in healthy subjects. These findings are important because they expand our understanding of dopaminergic modulation of inhibitory motor response control. Previous literature suggests a largely selective effect of D2-type receptors in motor inhibitory control processes (Groman and Jentsch, 2013, Jentsch et al., 2014), but findings from Study 1 indicates a distinct role for both striatal D1- and D2-type receptors that are specific to action-cancelation as assessed by the stop-signal task. Action-withholding capacity, as estimated by the continuous performance test, was not correlated with dopamine receptor BP$_{ND}$ suggesting it is governed by somewhat different neurocircuitry than action cancellation. In addition, the study demonstrated that the effect was selectively localized to the dorsal regions of the striatum, supporting this region as a key locus of dopaminergic control over motor response inhibition. The results from Study 1 support a growing literature highlighting the importance of the balance between striatal output signaling pathways in behavioral control.

Study 2 (Chapter 3) expanded upon these findings by examining trait-like impulsiveness, as assessed by several widely-used self-report personality scales, and the associations with D1-
and D2-type receptor BP_{ND}. The results show a negative correlation between D1-type receptors in the midbrain region and behavioral impulsivity as assessed by the BIS-11 (Reise et al., 2013). Although previous studies of personality scales of impulsiveness show associations with striatal dopaminergic signaling, there were no correlations involving striatal D1- or D2-type receptors and self-report scales in this study. Instead, the data suggest a role for dopamine transmission via D1-type receptors in the midbrain region in self-reported impulsivity. Taken together, the data from Study 1 and Study 2 provide evidence supporting the fundamental connection of dopaminergic signaling to inhibitory control, suggesting that greater striatal dopamine receptor availability is associated with lower levels of impulsivity. The findings advance the field by demonstrating a possible role for D1-type receptors in certain facets of human impulsivity. Furthermore the data align with a recent model describing the cooperative balance between D1- and D2-receptor mediated striatal output pathways to be essential for motor response control (Keeler et al., 2014).

However these studies also indicate differential dopaminergic involvement in trait-like forms of impulsivity than in state-like assessments of impulsive behavior. Self-report personality assessments are linked with dopamine receptors in the midbrain, while response inhibition task performance is associated with dopamine receptors in the dorsal striatum. These studies suggest that self-reported impulsivity and response inhibition may be rooted in different neurochemical processes, and support the functional separation of these assessments from a biological standpoint. While the complex balance of D1- or D2-type receptor mediated pathways in the striatum may be imperative to meet the task demands of motor response inhibition paradigms, this may not be physiologically relevant for the broader behavioral tendencies measured with self-report questionnaires. It is possible that stable expressions of trait-like impulsive
characteristics may be more influenced by transient inhibitory dopamine signaling via pre-synaptic or auto-receptor mechanisms in the midbrain. Midbrain D2-type receptors mediate the relationship between self-reported impulsiveness and striatal amphetamine-induced dopamine release, seemingly via auto-receptor mechanisms that down regulate dopaminergic striatal transmission (Buckholtz et al., 2010). Furthermore, midbrain D2-type BPND is linked to self-reported impulsiveness (Buckholtz et al., 2010) and related traits, like novelty seeking (Zald et al., 2008).

In broad terms, the studies presented here demonstrate an overall negative relationship between dopamine receptor availability and the impulsivity construct. Indicating that high levels of dopamine receptor availability confer low levels of impulsivity. These studies support the idea that down regulation of dopaminergic receptor availability (prior to drug use, or as a consequence of chronic drug use) may contribute to enhanced impulsivity observed in stimulant used disorder. These findings provide support for enhancing dopamine receptor availability as a treatment target for impulse control.

Although a large body of evidence suggests that methamphetamine dependence is associated with hypo-dopaminergic function there is little evidence demonstrating that the dopaminergic deficits can be ameliorated in human addicts. Therefore, study 3 (Chapter 4) examined the degree to which deficits in dopaminergic D2-type receptors can be reversed in methamphetamine dependent individuals with abstinence. Using individually tailored exercise programs, Study 3 demonstrated reversal of dopaminergic D2-type receptor deficits after 8-weeks in methamphetamine dependent subjects. This effect was shown to be selective to exercise over abstinence alone because no changes in D2-type receptor availability were observed in the
control group who participated in individually curated health-education programs showed. These findings are important because they demonstrate that the dopaminergic hypofunction associated with chronic methamphetamine use can be reversed in human addicts. The findings suggest that 8-weeks of drug abstinence maintained in a residential treatment setting is not sufficient to produce changes in D2-type receptors. However participation in an exercise regimen during that same period of abstinence can increase D2-type receptor levels. This finding is highly clinically relevant and supports the use of exercise as an adjunctive therapy for stimulant use disorder. Furthermore it increases the possibility of monitoring dopaminergic function during the course of addiction treatment as a means to inform on individual progression and biological response to therapeutics. These findings are important because the integrity of the frontostriatal circuitry is highly implicated in addictive disorders and treatment outcomes and any changes in this circuitry that occur with abstinence could be indicative of the normalization of frontostriatal deficits.

**GENERAL DISCUSSION**

Investigating the molecular correlates of impulsive behavior in healthy subjects helps define the biological basis of inhibition deficits in patients with substance use disorders. Furthermore, understanding the degree to which dopaminergic deficits in stimulant dependence can be normalized represents an important question in addiction treatment as dopamine system integrity play an important role in the etiology of addiction, but also in behaviors that are core to the addictive phenotype. Taken together, the studies presented in this dissertation provide evidence supporting a central role for striatal dopaminergic signaling in different dimensions of the impulsivity construct and emphasize a role for D1- and D2-type receptor signaling in these behaviors. These studies contribute to the understanding that different forms of impulsivity show different responses to pharmacological manipulation (Evenden, 1999, de Wit et al., 2002, Dalley
et al., 2008), different associations with biological markers (Robertson et al., 2015) and other cognitive constructs (Reynolds et al., 2006). Indicating that different forms of impulsivity likely depend upon distinct molecular mechanisms or separate neurocircuitry, supporting the separation of these constructs from a biological perspective.

Determining the neural substrates utilized during different forms of inhibition is important for the development of biological and cognitive markers that can be used to improve the differential diagnosis of neuropsychiatric disorders that tend to share common clinical symptoms. The use of biological or cognitive markers could increase treatment efficacy for these disorders by guiding approaches that are individually tailored to the patient’s cognitive and biological profiles. Furthermore, identifying the biological mechanisms underlying different forms of impulsivity and performance on neurocognitive tasks could improve clinical testing of therapeutic drugs or interventions, by identifying individuals with cognitive and biological profiles that are most likely to benefit. Furthermore, a robust understanding of the biological basis of inhibitory control could inform the selection of neurocognitive tasks and study outcome measures that are most appropriate for the therapeutic target under study.

The large degree of individual variance and patient heterogeneity are major challenges in the development of effective therapeutics or biological markers that are specific to certain neuropsychiatric disorders, especially substance use disorders. Although dopaminergic deficits are well established in groups of stimulant users relative to groups of healthy controls, there is a high degree of within-group variability. Individual variation in dopamine D2-type receptor availability contributes to varying effects receptor-specific agonists or antagonists. It is possible that some D2 agonists would improve inhibitory control in patients with impairments attributed
to D2-type receptor deficits, and improve addiction treatment outcomes in patients whose substance use behavior is rooted in lack of self-control and impulsiveness. However, different effects would be seen in patients with inhibition impairments derived from non-dopaminergic transmission (not D2-mediated).

The studies in this dissertation have limitations. Among them are the correlative designs of Study 1 and 2, which cannot inform on causal relationships between dopamine-receptor subtype signaling and inhibitory control or impulsiveness. Another is imperfect selectivity of the radioligands used. \[^{[11}C\]NNC-112 has approximately 10-fold higher \emph{in vivo} affinity for D1-type over 5HT2A receptors (Slifstein et al., 2007), and pharmacological blocking studies show that \~5\% of the \[^{[11}C\]NNC-112 signal in the striatum represents 5HT2A binding (Ekelund et al., 2007). Although contamination of the D1-receptor signal with 5HT2A binding is minor in the striatum, it should be acknowledged. \[^{[18}F\]fallypride has nearly equal affinity for D2 and D3 dopamine receptors \emph{in vivo} (Slifstein et al., 2004) and cannot distinguish between them; however, D2-type receptors in the dorsal striatum are almost exclusively D2 receptors with very low D3 expression (Murray et al., 1994). Thus, BP\textsubscript{ND} measurements in the dorsal striatum primarily reflect D2 receptor availability, and those in the ventral striatum are likely a combination of signal from D2 and D3 receptors. \[^{[18}F\]fallypride also binds to both isoforms of the D2 receptor (D2S and D2L); therefore, BP\textsubscript{ND} measurements using \[^{[18}F\]fallypride do not distinguish between pre- and post-synaptic D2 receptors. Furthermore, the cellular location and integrity of D2-type receptors bound to \[^{[18}F\]fallypride is uncertain. Presumably, membrane-bound receptors constitute the majority of the \[^{[18}F\]fallypride signal, however the degree to which these receptors are functionally active or located on the cell surface is unknown in these studies. It is possible that \[^{[18}F\]fallypride may show differential binding to functionally inactive or
intracellular D2-type receptors. Conversely, it is possible that $^{18}$F-fallypride could signal reflect receptor binding from a pool of behaviorally relevant D2-type receptors located at the cell surface and a pool of irrelevant D2-type receptors that are inactive or intracellular.

**Future Directions**

Future studies extending this research would benefit from the combined use of multiple PET assessments of the function of the dopaminergic system. Assessments of other neurotransmitter systems in combination would provide important information about the neurochemical interactions that underlie impulsivity. For example, the interaction of dopamine and serotonin systems is an important component in impulsive behavior, and a role for noradrenergic signaling is also emerging (Bari and Robbins, 2013). Moreover, multimodal neuroimaging studies employing MRI and PET imaging methods could provide data to determine the molecular correlates of neural activation (as indexed by BOLD signal) that is specific to different forms of inhibition. Further MRI techniques can detect differences in the neural circuitry recruited for task performance despite a lack of differences in the behavioral measures, which could be useful in identifying early perturbations in the frontostriatal system before the emergence of behavioral deficiencies.

Neuroimaging techniques have played a significant role in advancing our understanding the importance of impulsivity in addictive disorders. However, there remains much to be learned about the predictive value of impulsivity and inhibitory towards risk-related behaviors in the future and the effectiveness of prevention strategies against drug use. We have yet to understand how neurobiological markers (e.g. of dopamine function etc.) confer risk for addictive disorders or other neuropsychiatric illness, or response to treatment. However, future work focusing on
techniques clinicians and care providers can use to incorporate this information to guide treatment approaches is essential to improving care for those afflicted with neuropsychiatric disorders.
References for Chapter 5

Bari A, Robbins TW (2013) Noradrenergic versus dopaminergic modulation of impulsivity, attention and monitoring behaviour in rats performing the stop-signal task: possible relevance to ADHD. Psychopharmacology (Berl) 230:89-111.


