

ASSESSMENT OF THE DOPAMINE SYSTEM IN ADDICTION  
USING POSITRON EMISSION TOMOGRAPHY

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Submitted to the faculty of the University Graduate School  
in partial fulfillment of the requirements  
for the degree  
Doctor of Philosophy  
in the Program of Medical Neuroscience,  
Indiana University

February 2014

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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## Acknowledgements

This work was supported by: funding to KKY from the IUSM Department of Radiology and Imaging Sciences; supplementary NIDA funding to 1R21DA023097-01A1 (PDS) and the Brain and Behavior Research Foundation (NARSAD; PDS); ABMRF/The Foundation for Alcohol Research (KKY), NIAAA 5P60AA007611-25 (pilot P50 to KKY), NIAAA R21AA016901 (KKY), NIAAA R01AA018354 (KKY); the Indiana Clinical and Translational Sciences Institute (NIH TR000006, Indiana Clinical Research Center); and the Indiana University-Purdue University at Indianapolis Research Support Funds Grant (KKY). In addition, support for education for Mr. Albrecht was provided by the IUSM Integrated Biomedical Gateway Program, the Stark Neurosciences Research Institute, and the Department of Radiology and Imaging Sciences at the IU School of Medicine.

The author would also like to thank Dr. David E. Moody and Dr. David Andrenyak for performing the THC, OH-THC, and THC-COOH analysis (Chapter 1), which was supported by NIDA contract N01DA-9-7767 to PDS.

The author gratefully acknowledges the assistance and support of his primary mentor, Karmen K. Yoder, PhD, as well as the other members of his dissertation committee: Gary D. Hutchins, PhD (chair); Andrew J. Saykin, PsyD; David A. Kareken, PhD; and Nicholas J. Grahame, PhD. Additionally, the author would like to thank the following individuals for their assistance and support with the included works, as well as related assistance throughout the author's graduate career: Vivian Arnold; Michele Beal; Clive Brown-Proctor, PhD; Margaret Brumbaugh; Jenya Chumin; Susan Conroy, PhD; Joey Contreras; Larry Corbin, AS; Cari Cox-Lehigh; Ted Cummins, PhD; Cristine Czachowski, PhD; Mario Dzemidzic, PhD; William Eiler, PhD; Lauren Federici; Tatiana Foroud, PhD; Dennise Garzon, MS; Barbara Glick-Wilson, PhD; Tammy Graves; Mark Green, PhD; Christine Herring; Karen Hile; Cynthia Hingtgen, PhD; Mark Inlow, PhD; Brenna McDonald, PhD; Bruce Mock, PhD; Evan Morris, PhD; Morgan Mrotek; Marc Normandin, PhD; Grant Nicol, PhD; Brandon Oberlin, PhD; Elizabeth Patton; Kevin Perry; Shannon Risacher, PhD; Courtney Robbins; John Schild, PhD; Patrick Skosnik, PhD; Brandon Steele; Jenna Sullivan, PhD; Jennifer Vollmer; James Walters, MS; John West, PhD; Xin Zhang, PhD; Qi-Huang Zheng, PhD.

Finally, the author thanks his family and friends for their love and support, without which the undertaking resulting in this body of work would not have been possible. The author thanks his parents: John Albrecht and Ellen Nicholas Albrecht, siblings: Wendy

Albrecht, Alex Albrecht, Abby Albrecht, Jillian Albrecht, and Greg Odgen, and his beautiful nephew and nieces: Simon, Vivianne, and Annika, for their unconditional love and support.

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EMISSION TOMOGRAPHY

Drug addiction is a behavioral disorder characterized by impulsive behavior and continued intake of drug in the face of adverse consequences. Millions of people suffer the financial and social consequences of addiction, and yet many of the current therapies for addiction treatment have limited efficacy. Therefore, there is a critical need to characterize the neurobiological substrates of addiction in order to formulate better treatment options. In the first chapter, the striatal dopamine system is interrogated with [<sup>11</sup>C]raclopride PET to assess differences between chronic cannabis users and healthy controls. The results of this chapter indicate that chronic cannabis use is not associated with a reduction in striatal D<sub>2</sub>/D<sub>3</sub> receptor availability, unlike many other drugs of abuse. Additionally, recent cannabis consumption in chronic users was negatively correlated with D<sub>2</sub>/D<sub>3</sub> receptor availability. Chapter 2 describes a retrospective analysis in which striatal D<sub>2</sub>/D<sub>3</sub> receptor availability is compared between three groups of alcohol-drinking and tobacco-smoking subjects: nontreatment-seeking alcoholic smokers, social-drinking smokers, and social-drinking non-smokers. Results showed that smokers had reduced D<sub>2</sub>/D<sub>3</sub> receptor availability throughout the striatum, independent of drinking status. The results of the first two chapters suggest that some combustion product of marijuana and tobacco smoke may have an effect on striatal dopamine concentration. Furthermore, they serve to highlight the effectiveness of using baseline PET imaging to characterize dopamine dysfunction in addictions. The final chapter explores the use of [<sup>18</sup>F]fallypride PET in a proof-of-concept study to determine whether changes in cortical dopamine can be detected during a response inhibition task. We were able to detect several cortical regions of significant dopamine changes in response to the task, and the amount of change in three regions was significantly associated with task performance. Overall, the results of Chapter 3 validate the use of [<sup>18</sup>F]fallypride PET to detect cortical dopamine changes during a impulse control task. In summary, the results reported in the current document demonstrate the effectiveness of PET imaging as a tool for probing resting and activated dopamine systems in addiction. Future studies will expand on these results, and incorporate additional methods to further elucidate the neurobiology of addiction.

Gary D. Hutchins, PhD, Chair

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## List of Abbreviations

$\Delta BP_{ND}$	change in binding potential
$^{\circ}C$	degrees celcius
3T	3 Tesla
AA	African American
ACC	anterior cingulate cortex
AI	anterior insula
ANOVA	analysis of variance
A-O	action-outcome
ATM	atomoxetine
AUC	area under the curve
AUDIT	Alcohol Use Disorder Identification Test
BAES	Biphasic Alcohol Effects Scale
BIS-11	Barratt Impulsivity Scale
BOLD	blood oxygen level dependent
$BP_{ND}$	binding potential
BrAC	breath alcohol
C	Caucasian
CAN	cannabis
CB1	cannabinoid type 1
cc	cubic centimeter
CIWA-Ar	Clinical Withdrawal Assessment for Alcohol, Revised
COMT	catechol-O-methyltransferase
CON	control
Cr	creatinine
CWS	Cigarette Withdrawal Scale
DA	dopamine
Daergic	dopaminergic
DCA	dorsal caudate
dL	deciliter
DLS	dorsolateral striatum
DMS	dorsomedial striatum

DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
DTI	diffusion tensor imaging
EtOH	ethanol
FAL	[ <sup>18</sup> F]fallypride
FLU	α-flupenthixol
fMRI	functional magnetic resonance imaging
FSCV	fast-scan cyclic voltammetry
GC-MS	gas chromatography-mass spectrometry
GP	globus pallidus
I	Asian-Indian American
IFC	inferior frontal cortex
IFG	inferior frontal gyrus
IPL	inferior parietal lobule
ITG	inferior temporal gyrus
IV	intravenous
kg	kilogram
L	left
LSD	least square difference
MAO-A	monoamine oxidase A
MAO-B	monoamine oxidase B
MBq	megabecquerel
mCi	millicurie
MFG	middle frontal gyrus
mg	milligram
mL	milliliter
mm	millimeter
MNI	Montreal Neurological Institute
MP	methylphenidate
MP-RAGE	magnetized prepared rapid gradient echo
MRI	magnetic resonance imaging
MRTM	multilinear reference tissue model
N/A	not applicable
NAcc	nucleus accumbens
NE	norepinephrine

ng	nanogram
NHL	Non-Hispanic Latino
NIfTI	Neuroimaging Informatics Technology Initiative
nmol	nanomole
NTS-S	nontreatment-seeking smokers
OFC	orbitofrontal cortex
OH-THC	11-hydroxy-THC
PAS	Perceptual Aberration Scale
PET	positron emission tomography
PFC	prefrontal cortex
postDCA	postcommissural dorsal caudate
postDPU	postcommissural dorsal putamen
preDCA	precommissural dorsal caudate
preDPU	precommissural dorsal putamen
pre-SMA	presupplementary motor area
PUT	putamen
QC	quality control sample
R	right
RAC	[ <sup>11</sup> C]raclopride
rCBF	regional cerebral blood flow
ROI	region of interest
RS-fMRI	resting state fMRI
RT	reaction time
SCID	Structured Clinical Diagnostic Interview for DSM-IV Disorders
SD	standard deviation
SD-NS	social-drinking non-smokers
SD-S	social-drinking smokers
SFG	superior frontal gyrus
SMA	supplementary motor area
SMG	supramarginal gyrus
SN	substantia nigra
SPECT	single photon emission computed tomography
SPM	Statistical Parametric Mapping
SPQ	Schizotypal Personality Questionnaire

S-R	stimulus-response
SSAGA	Semi-Structured Assessment for the Genetics of Alcoholism
SSD	stop signal delay
SSRT	stop signal reaction time
SST	stop signal task
STG	superior temporal gyrus
STN	subthalamic nucleus
THC	$\Delta$ 9-tetrahydrocannabinol
THC-COOH	11-nor- $\Delta$ 9-THC-9-carboxylic acid
TLFB	Time Line Follow Back
TPQ	Tridimensional Personality Questionnaire
VST	ventral striatum
VTA	ventral tegmental area

## Introduction

Drug addiction is a behavioral disorder characterized by impulsive behavior and continued intake of drugs in the face of adverse consequences. Substance abuse is a ubiquitous global phenomenon. In 2011, in the U.S. alone, 38 million people age 12+ reported illicit drugs use, 81 million reported tobacco use, and 168 million reported alcohol use (Samhsa, 2011). However, only a relatively small proportion of people who engage in recreational substance use will progress to addiction. In 2011, 11.2 million Americans met DSM-IV criteria for alcohol or illicit drug dependence, and 32 million met criteria for nicotine dependence (Samhsa, 2011). The economic impact from these addictions is staggering; estimates have placed the annual economic cost of alcohol, tobacco, and illicit drug abuse to society in the U.S. at 191.6, 167.8, and 151.4 billion dollars, respectively (Fellows, 2002; Harwood, 2001; Harwood, 2000). The impact of addictions on health care is similarly massive. A comprehensive study of the global health burden from addictions reported that alcohol and tobacco were each responsible for 4% of the global disease burden (as measured by disability adjusted life years), and illicit drugs were responsible for 0.8% of the burden (Rehm, 2006).

One characteristic shared by many addicts is the inability to abstain from substance abuse, despite a desire to quit. The vast majority of addicted individuals will attempt, often unsuccessfully, to discontinue substance abuse at some point during their use period. There is a high likelihood of relapse during a period of attempted abstinence, and relapse rates are similar among the various substances of abuse (Hunt, 1971). Relapse rates average around 80% for alcohol (Miller, 1996); 95% for tobacco (Hughes, 2004); and 85% for heroin (Darke, 2005). There are many different treatment methods for addiction, and most fall into the categories of either behavioral or pharmacological therapies (Dutra, 2008; O'brien, 2006). Even though substantial resources have gone into developing and optimizing new treatments, efficacies remain modest. A better understanding of the neurobiology of addiction would provide a framework for the development of more effective therapies. Therefore, there is a critical need to elucidate the neurobiological substrates underlying the process of addiction.

### *Dopaminergic Circuitry and Signaling*

Dopamine (DA) is a monoamine neurotransmitter that operates on a wide variety of brain functions. The primary DA-producing nuclei in the brain are the ventral

tegmental area (VTA) and substantia nigra (SN), located in the midbrain (Swanson, 1982). Three main afferents arise from the VTA/SN: the mesolimbic circuit, which originates from VTA neurons and projects to the ventral striatum; the nigrostriatal circuit, which originates from the SN and projects to more dorsal striatal regions; and the mesocortical circuit, which originates from the VTA and projects to the frontal cortices. Projections from the midbrain to the striatum are arranged in an inverted topography, such that the dorsal most nuclei project more ventrally, and the ventral most nuclei project more dorsally (Fallon, 1978). Although early tracing studies seemed to indicate that these circuits were distinct in their terminal projection fields, more recent research has uncovered a substantial amount of overlap in VTA/SN projection fields (Lynd-Balta, 1994a, 1994b). Overlapping projection areas allow for greater modulation of dopaminergic (DAergic) signaling, as neurotransmission is under control of more than one region. Furthermore, for each projection from the midbrain nuclei to the striatum, there are two reciprocal projections back to the midbrain: one that forms a “closed” loop with the originating midbrain projection area, and one that synapses in a midbrain region laterally to the origin (Haber, 2000). In this manner, activity in the ventral striatum can affect activity in the dorsal striatum, although there are no direct connections between the regions. Similarly to midbrain innervation of the striatum, corticostriatal projections are also arranged topographically, and the projection fields of distinct cortical regions overlap in their striatal terminals (Haber, 2006).

DA neurons of the SN/VTA display two different types of firing patterns: tonic and phasic. Phasic and tonic DA signaling can be modulated differently, depending on the type of afferent input (Floresco, 2003). Neurons in a freely moving animal can switch between tonic and phasic signaling, and firing rates have been shown to increase in response to environmental salient stimuli (Hyland, 2002). Importantly, DA-dependent behaviors can be differentially regulated by tonic or phasic signaling (Zweifel, 2009). In this elegant study by Zweifel et al., phasic DA signaling was selectively ablated in order to assess regulation of behavior by phasic or tonic DAergic transmission. Disruption of the phasic DA transients impaired behaviors that involved learned associations of environmental cues with salient events. Many other behaviors were unaffected, presumably because of the functionally intact tonic DA signaling. In this manner, drugs that effect phasic and tonic DA signaling disparately may have correspondingly different effects on behavioral outcome.

DA binds to two different classes of G-protein coupled receptors in the brain: D<sub>1</sub>-like [D<sub>1</sub> and D<sub>5</sub>], and D<sub>2</sub>-like [D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>] (Vallone, 2000). DA receptors are widely expressed throughout the brain, with the highest expression levels in the striatum (containing the caudate, putamen, and nucleus accumbens), olfactory tubercle, amygdala, and SN/VTA (Jackson, 1994). Receptor expression is relatively moderate in other regions, i.e. cerebral cortex, hippocampus, thalamus, and cerebellum. It is no coincidence that many of these structures are heavily involved in the neurobiology of addiction. DA modulates a wide array of addictive processes via complicated signaling between these structures.

#### *Involvement of DA throughout Addiction*

One of the initial findings suggesting the involvement of DA in addiction was the ability of drugs of abuse to increase extracellular DA concentrations in the nucleus accumbens of rats (Di Chiara, 1988b). This pioneer study collected dialysate from rat nucleus accumbens (NAcc) and dorsal caudate (DCA) during investigator-administered drug challenges with both common drugs of abuse (opiates, ethanol, nicotine, amphetamine, and cocaine) and non-abused drugs (haloperidol, imipramine, atropine, and diphenhydramine). DA release varied across substances; the non-abused drugs imipramine, atropine, and diphenhydramine elicited no detectable DA release in either brain region; haloperidol, a neuroleptic with DA antagonist actions, increased basal DA equally in the NAcc and DCA; all of the abused drugs also increased extracellular DA concentrations in both regions, but the magnitude of NAcc release was significantly higher than release in the DCA. Subsequent studies replicated these findings and extended them to include cannabinoids (Kalivas, 1990; Tanda, 1997a; Yoshimoto, 1992).

While studies of investigator-administered drugs are useful, an animal model of addiction that incorporates voluntary substance intake is more closely related to the human condition. Many studies of drug-induced DA release incorporate a self-administration paradigm, where an animal is trained to perform a specific type of response to earn access to the drug (for a comprehensive review, see Gardner, 2000). Self-administration studies have reported DA release in the NAcc during voluntary intake of cocaine, ethanol, heroin, and cannabinoids (Di Ciano, 1995; Fadda, 2006a; Pettit, 1989; Weiss, 1993), similar to studies of investigator-administered drugs. For the sake of brevity, this discussion of drug-induced DA release has been presented in a simplistic



light, as variables such as cellular mechanisms and timing of DA release are outside the scope of the current discussion. Together, results from these studies led to the conclusion that all drugs of abuse share the characteristic of preferentially increasing extracellular DA in the NAcc, at least during initiation of use (the time course of DA signaling during addiction will be discussed in the next section).

It was originally thought that drug-induced DA release in the ventral striatum (VST) was directly responsible for the subjective euphoria associated with drugs of abuse (Wise, 1978), but the role of DA in reward is likely more complicated. A sophisticated series of electrophysiological studies helped shed light on this issue (Schultz, 1997). When animals were presented with an unpredicted rewarding stimulus that was temporally paired with a visual or audio cue, DA neurons responded by burst-firing. This increased firing presumably increased extracellular DA concentrations in the NAcc, as might have been predicted by the drug administration studies above. However, after repeated pairing of cue and reward, the DA neurons began firing after presentation of the conditioned cue, but not during presentation of the reward. This shift in firing patterns indicated that DA neurons were not responding to the reward itself, but rather to some learned association between the cue and reward. Additionally, the DA neurons displayed decreased firing if the conditioned cue was presented, but no reward delivered. The authors deemed this a “prediction error” signal. Although a discussion of the prediction error hypothesis is outside the scope of this document, the role of DA in cue conditioning is an important one, as is the concept of temporally dynamic DA signaling.

While it is generally accepted that most drugs of abuse share the ability to acutely increase DA concentrations in the ventral striatum, this is not sufficient to cause addiction. Indeed, only an estimated 2.9% of individuals that try addictive substances in their life will proceed to addiction (Grant, 1998). Therefore, there must be additional neurobiological factors that contribute to the progression to addiction. It has been suggested that substance intake begins as a goal-directed behavior (mediated by action-outcome (A-O) associations), that progresses to habitual substance intake (mediated by stimulus-response (S-R) associations), over the course of addiction (Everitt, 2001). Goal-directed (A-O) behaviors are governed by an associative representation of the contingency between the action and the outcome. Relative to human substance intake, individuals initiate drug taking behavior because of the association between the substance and its rewarding effects. In contrast, habitual (S-R) behaviors are simple

response habits that are triggered by environmental stimuli, whereby presentation of a stimulus will reliably and automatically elicit a response, even without contingent presentation of a reward. Relative to human drug use, this is akin to the initiation of drug-seeking behavior following exposure to a drug-associated stimulus. Based on these concepts, it is possible to experimentally assess whether a behavior is more under goal-oriented or habitual control. Because A-O behaviors depend on the relationship between action and reward outcome, changing that association will lead to behavioral changes (e.g. devaluing the reward will lead to fewer lever presses in the drug-seeking stage) (Adams, 1981). Conversely, if a behavior is habitual (S-R), then devaluing the relationship between action and reward outcome will not affect drug-seeking behavior. Using this devaluation paradigm, researchers have been able to gain important information about the progression of addiction from the initiation of drug-taking to compulsive drug abuse.

Evidence indicates that initial goal-directed behavior is mediated largely by VST (NAcc) and dorsomedial striatum (DMS), whereas habitual, compulsive behavior is mediated more so by dorsolateral striatum (DLS). Inactivation of the NAcc (Corbit, 2001; Kelley, 1997) or DMS (Yin, 2005) impairs instrumental behavior under action-outcome control, as rewards become insensitive to devaluation. Conversely, DLS-lesioning impairs habit formation, and shifts behavior towards more action-outcome control (Yin, 2005). Furthermore, recent studies suggest that the shift from goal-oriented behavior to habitual behavior throughout the course of addiction is accompanied by a shift in behavioral control from VST to DMS to DLS. Initial drug use is under control of goal-directed behavior, which is supported by animal models of reward devaluation in early cocaine and ethanol seeking (Corbit, 2012; Olmstead, 2001; Samson, 2004; Zapata, 2010), [although there is some evidence that different types of instrumental training produce habitual behavior prematurely (Dickinson, 2002; Mangieri, 2012; Murray, 2012)]. However, after extended training periods, drug-seeking cannot be attenuated by reward devaluation, indicating a transition from action-outcome to habitual responding (Corbit, 2012; Zapata, 2010). Inactivation of the DMS during acquisition of early alcohol-seeking impairs goal-oriented behavior (Corbit, 2012). Conversely, when drug-seeking is under habitual control after extensive training, inactivation of the DMS has no effect, whereas DLS inactivation alters habitual responding and reverts behavior to goal-directed control, reinstating sensitivity to reward devaluation (Corbit, 2012; Zapata, 2010).

Importantly, this signaling shift between striatal regions is regulated, in part, by DA. During acquisition of early cocaine-seeking, intra-cranial infusion of  $\alpha$ -flupenthixol (FLU; a non-selective DA antagonist) into the DMS, but not DLS, disrupted goal-directed cocaine-seeking (Murray, 2012). Conversely, intra-cranial infusion of FLU into the DLS, but not DMS, caused aberrant drug-seeking after habitual responding had been established (Belin, 2008; Murray, 2012). Furthermore, the transition from ventral to dorsal striatal areas during the progression of drug use is dependent on serial connectivity linking the ventral to dorsal striatum (Belin, 2008; Willuhn, 2012). Belin et al. (2008) showed that a unilateral NAcc lesion attenuated drug-seeking to the same degree as intra-DLS FLU infusions, indicating that intact NAcc signaling is necessary for the development of DLS-controlled habitual behavior. Using fast-scan cyclic voltammetry (FSCV), Willuhn et al. (2012) examined phasic DA signaling during progression of cocaine self-administration that was paired with conditioned stimuli. They replicated the cocaine-induced phasic DA release in the VST, and demonstrated that the DA release gradually decreased in magnitude over three weeks. In an opposite manner, DA release in the DLS was absent during the acquisition of cocaine intake, but significantly increased over the three weeks. The VST was then lesioned unilaterally, which left DA signaling intact in the contralateral DLS. However, DA transients in the DLS ipsilateral to the VST lesion were completely ablated, and absent throughout three weeks of cocaine use. Based on this evidence, the ventral to dorsal shift in locus of behavioral control is thought to be dependent on the spiraling connections between ventral and dorsal striatum (Haber, 2000). Taken together, the above studies strongly advance the idea that initial involvement of ventral striatal DA transmission in drug taking gradually progresses to more dorsal striatal regions, eventually resulting in compulsive drug intake.

#### *Human PET Imaging in Addiction*

Small animal studies, such as those mentioned in the above section, have been instrumental in understanding basic DAergic contributions to addiction. However, addiction is a purely human affliction, and knowledge gained from small animal studies must be applied to the human condition with this caveat in mind. Therefore, studies in human addicts are necessary to understand the role of DA in addiction in a more representative sample. Because methods used to investigate neurochemistry in animals are far too invasive to be used ethically in humans (e.g. microdialysis, voltammetry), a

relatively non-invasive technique like positron emission tomography (PET) imaging offers unique advantages. PET relies on systemic administration of radioligands (or radiotracers) that travel to the brain and bind to a molecule of interest. Radioligands exist for several neurotransmitter systems, but this discussion will focus on DA receptor-specific ligands. Detection and localization of radioactive decay events by a PET scanner allows for *in vivo* characterization of human DA systems. The most commonly used quantitative outcome in PET imaging is “binding potential” ( $BP_{ND}$ ).  $BP_{ND}$  is operationally defined as  $B_{avail}/K_D$ , where  $B_{avail}$  refers to the density of receptors available to bind radioligand *in vivo*, and  $K_D$  refers to the radioligand equilibrium dissociation constant (Innis, 2007).  $BP_{ND}$  can be used to estimate DA receptor expression levels, but it can also be sensitive to levels of endogenous DA, as intrasynaptic and extracellular DA can compete with radioligands for binding at the receptor. The susceptibility of radioligand binding to competition from endogenous DA varies widely for a number of radioligands (for a comprehensive review of this topic, see Yoder, 2011c). The most commonly used radioligands for imaging the DA system are [ $^{11}C$ ]Raclopride (RAC), [ $^{18}F$ ]Fallypride (FAL), [ $^{11}C$ ]FLB, and [ $^{11}C$ ]PHNO, and many of the studies discussed in later sections employ these ligands. It is important to note that the radiotracers listed above all specifically bind D<sub>2</sub>-like receptors. There are many tracers that specifically target D<sub>1</sub>-like receptors (Laruelle, 2000), but the current discussion will focus on D<sub>2</sub>-specific tracers. Because some tracers are able to be displaced by endogenous DA, they can be used to detect DA release *in vivo* in response to some sort of challenge (e.g. pharmacologic, cognitive) (Dewey, 1991; Dewey, 1993). Based on these properties, DA-specific radiotracers have been used to assess both differences in baseline D<sub>2</sub> receptor availability associated with addiction, as well as DA release in response to pharmacological drug effects, or conditioned properties associated with certain drugs. The following sections will include a critical review of studies that have employed these techniques to investigate DAergic function in substance addicts.

### *Baseline Differences in Striatal D<sub>2</sub> Receptors- association with Addiction*

Since the initial development of PET radioligands specific to D<sub>2</sub>-like receptors, an immense amount of work has examined baseline striatal D<sub>2</sub> receptor availability differences between addicted populations and healthy controls. As will be discussed, there is a high degree of variability between study results. However, this undertaking has yielded a great body of information regarding how DAergic systems are affected in

addiction. Many of these studies reported lower D<sub>2</sub> receptor availability in individuals that are dependent on or heavy users of alcohol (Heinz, 2004; Hietala, 1994; Martinez, 2005; Rominger, 2012b; Volkow, 2007; Volkow, 1996), cocaine (Martinez, 2004; Martinez, 2011; Martinez, 2009; Volkow, 1997), opiates (Martinez, 2012; Wang, 1997a; Zijlstra, 2008), methamphetamines (Lee, 2009; Volkow, 2001a), and tobacco (Albrecht, 2013; Busto, 2009; Fehr, 2008; Stokes, 2012), relative to healthy controls. Conversely, no investigations of chronic cannabis users have reported any differences in striatal D<sub>2</sub> receptor availability between this population and healthy controls (Albrecht, 2012a; Sevy, 2008b; Stokes, 2012; Urban, 2012b).

Though this body of work and the accordance of results are impressive, there are a number of similar studies that reported contrary findings for certain drug classes. Using SPECT D<sub>2</sub>-binding ligands [<sup>123</sup>I]epidepride and [<sup>123</sup>I]IBZM, and PET ligands FAL and RAC, several groups reported no differences in striatal D<sub>2</sub> receptor availability between alcoholics and controls (Albrecht, 2013; Guardia, 2000b; Repo, 1999b; Spreckelmeyer, 2011). It is possible that some discrepancies result in part from the use of SPECT tracers (Guardia, 2000b; Repo, 1999b), whereas the studies reporting significant differences utilized mainly RAC and FAL. However, another possible explanation lies in differential matching of control subjects. Many studies of addicted populations exclude subjects for substance use, but often with the exception of tobacco cigarettes. Because significant differences in D<sub>2</sub> receptor availability have been associated with tobacco smoking (see above), it is possible that imbalances in smoking status between alcoholics and controls in some studies may have accounted for reports of lower D<sub>2</sub> receptor availability in alcoholics (Heinz, 2004; Hietala, 1994; Rominger, 2012b; Volkow, 2007; Volkow, 1996). Interestingly, of the two studies that used FAL to estimate baseline D<sub>2</sub> availability in alcoholics, the one that matched for smoking status in controls reported no effects (Spreckelmeyer, 2011), while the one that did not match for smoking status reported lower striatal FAL BP<sub>ND</sub> in alcoholics (Rominger, 2012b). Although this point strongly supports careful matching of controls to addicted individuals, one investigation matched controls for smoking status and reported lower striatal D<sub>2</sub> availability in alcoholics (Martinez, 2005). Only two studies in alcoholics have both used RAC and matched controls for smoking status, and reported contrasting results (Albrecht, 2013; Martinez, 2005). Differences in alcoholic populations in these two studies, [nontreatment-seeking alcoholics in (Albrecht, 2013) and detoxified and

abstinent alcoholics in (detoxified and abstinent alcoholics in Martinez, 2005)], could potentially account for the discrepant results.

Similar to the story for alcohol, one group reported that tobacco-smoking subjects did not display lower striatal D<sub>2</sub> receptor availability compared to non-smoking controls (Yang, 2006). The main difference between the study by Yang et al. (2006) and those reporting positive results, is that the former study used SPECT and [<sup>123</sup>I]IBZM to estimate D<sub>2</sub> availability. It is possible that SPECT methodology may not be best suited for detecting effects of group (see above, Guardia, 2000b).

Based on results from the above studies, there is a strong consensus that heavy abuse of psychostimulants (cocaine, methamphetamine), opiates, and tobacco (though some effects of tobacco may be sex-linked, Brown, 2012) is associated with lower striatal D<sub>2</sub> receptor availability. Chronic use of alcohol also appears to be associated with lower striatal D<sub>2</sub> availability (Martinez, 2005), and this effect may be most apparent in severe alcoholism. It is unclear whether lower striatal D<sub>2</sub>/D<sub>3</sub> receptor availability occurs prior to the onset of substance abuse, or is a consequence of long-term abuse. There is some evidence to suggest that higher D<sub>2</sub> receptor availability is protective in unaffected family members of alcoholics (Volkow, 2006a), but unfortunately cross-sectional studies are ill equipped to distinguish this difference. In contrast to other drugs of abuse, there have been no findings of lower striatal BP<sub>ND</sub> in cannabis users. Investigations comparing striatal D<sub>2</sub> receptor availability in addicted populations should incorporate careful matching of control subjects in the study design.

### *Drug-induced DA Release in Human Subjects*

As discussed previously, an abundance of animal studies have confirmed that virtually every drug of abuse elicits increases in extracellular DA levels, but the evidence for DA release in response to certain drug classes is less conclusive in human studies. There is a consensus among many studies that psychostimulants (e.g. cocaine, amphetamine, methylphenidate) reliably increase striatal DA in both healthy controls and addicted populations (Cox, 2009; Drevets, 2001; Martinez, 2012; Martinez, 2003; Oswald, 2007; Schlaepfer, 1997; Urban, 2012b; Volkow, 2007). Therefore, the following discussion will be limited to studies employing non-psychostimulant challenges. Because DA release in response to drug intake is thought to play an important role in the development and maintenance of addiction, studies of drug-induced DA release in

humans can yield critical information about the DAergic response to specific substances of abuse.

Several RAC PET studies have attempted to detect DA release in social-drinkers via an alcohol challenge, with varying results. Four studies used oral alcohol in order to induce striatal DA release (Boileau, 2003; Salonen, 1997; Setiawan, 2013; Urban, 2010). All these studies, with the exception of Salonen et al. (1997), reported significant decreases in striatal RAC  $BP_{ND}$ , indicative of DA release in response to the alcohol. Specifically, Boileau et al. (2003) found DA release in the ventral striatum (VST) in six social-drinking males; Urban et al. (2010) reported significant DA release in all striatal regions in men, but only in VST and precommissural dorsal putamen (preDPU) in women; and Setiawan et al. (2013) found that the direction of DA change in response to alcohol was dependent on subjective response to intoxication, where high responders displayed decreased DA after alcohol and low responders displayed increased DA. Conversely, in four separate studies that utilized an IV alcohol challenge (Ramchandani, 2011; Yoder, 2007; Yoder, 2005; Yoder, 2009), only one reported significant DA release in the VST, but only after alcohol delivered unexpectedly (Yoder, 2009). Specifically, two studies from Yoder et al. ('05, '07) found no group effect of alcohol on RAC  $BP_{ND}$ ; Ramchandani et al. (2011) reported a significant effect of *OPRM1* genotype on striatal DA release, but no significant within-group effects of alcohol. The inconsistent results from these studies suggest that the different modalities of alcohol presentation (oral vs. IV) might account for the different reports of DA release. Importantly, conditioned cues associated with drug intake might be important in modulating the DA response (this topic will be discussed in detail later).

Regardless of alcohol administration method, several of these studies also reported correlations between the magnitude of DA release and the subjective effects of alcohol. Urban et al. (2010) reported a significant positive correlation between change in activation on the biphasic alcohol effects scale (BAES) and VST  $\Delta BP_{ND}$  across all subjects. None of the other oral alcohol studies reported such correlations, though one did state that self-reported impulsiveness was a significant predictor of VST  $\Delta BP_{ND}$  (the direction of this association was unclear, Boileau, 2003). Two studies by Yoder et al. reported positive correlations between subjective intoxication and change in DA after IV alcohol. In one, the magnitude of DA release (number of voxels with  $\Delta BP_{ND} > 0$ ) was positively related to intoxication (Yoder, 2007), and in the other  $\Delta BP_{ND}$  in the left anterior putamen was associated with peak intoxication score (Yoder, 2005). However, in the

latter study, the authors cautioned careful interpretation of the relationship, as it also included negative  $\Delta BP_{ND}$  values, such that both increases and decreases in DA contributed to the correlation with changes in intoxication. It is interesting to note that the only study of IV alcohol that reported significant alcohol-induced changes in DA in response to unexpected alcohol did not find any associations with subjective effects of alcohol (Yoder, 2009). This fact lends support to the authors' suggestion that the changes in DA resulted from violations of reward expectation rather than pharmacologic effects of alcohol, thus the dissociation between changes in DA and the subjective experience of intoxication.

Similarly to alcohol, a large number of studies have investigated DA release in response to a nicotine or cigarette challenge in chronic smokers. By and large, these studies have indicated that smoking a tobacco cigarette induces striatal DA release. The majority of these reported smoking-induced DA release preferentially in the VST (Brody, 2010; Brody, 2009; Brody, 2006; Brody, 2004; Le Foll, 2013; Scott, 2007), but some studies using a voxel-wise analysis method reported DA release in dorsal aspects of the striatum as well (Domino, 2012; Domino, 2013). Several of these studies attempted to separate the pharmacologic effects of nicotine from the chemosensory cues associated with cigarette smoking by including a condition where subjects also smoked denicotinized cigarettes (usually containing  $< 0.1$ mg nicotine, compared to  $\sim 1$ mg in a regular cigarette). Though each of these studies reported greater DA release during smoking of a regular cigarette compared to a denicotinized cigarette, there were inconsistencies in the variables analyzed across studies. Two of these studies compared only DA release (indicated by  $\Delta BP_{ND}$ ) between scans during smoking of either a regular cigarette or denicotinized cigarette, but reported no significant within-condition effects (Brody, 2009; Scott, 2007). The other two compared only  $BP_{ND}$  after regular or denicotinized cigarette smoking, rather than differences in  $\Delta BP_{ND}$  between conditions (Domino, 2012; Domino, 2013). This method may have failed to account for baseline  $BP_{ND}$  differences between the two conditions. Additionally, only one of these studies analyzed DA release specifically during smoking of a denicotinized cigarette (pre-denicotinized  $BP_{ND}$  vs. post-denicotinized  $BP_{ND}$ ), and reported significant increases in dorsal striatal DA (Domino, 2013). As mentioned previously, this may indicate a DAergic response specifically to conditioned cues involved with smoking, but in the absence of nicotine (to be discussed later in the Introduction). To further investigate the pure pharmacological effects of nicotine on DA transmission, several studies examined DA



release in response to intranasal nicotine or nicotine gum. Two of these reported no significant intranasal nicotine-induced striatal DA release in humans (Montgomery, 2007) and non-human primates (Tsukada, 2002), but one claimed that nicotine gum induced striatal DA release compared to placebo gum (Takahashi, 2008). In the latter study, it is possible that the placebo gum condition induced a negative prediction error effect in smokers, as nicotine was expected but not delivered. In this manner, their findings could be a result of decreased DA during the placebo condition rather than increased DA during the nicotine condition.

Additionally, many of these studies reported associations between smoking-induced DA release and subjective variables. Several studies reported associations of DA release with changes in subjective craving, such that greater magnitudes of DA release were related to greater decreases in craving (Brody, 2006; Brody, 2004; Le Foll, 2013). However, only Le Foll et al. ('13) indicated a unidirectional relationship, whereby only positive changes in  $BP_{ND}$  were associated with reduced craving. The extent of the relationship between DA release and craving in the studies from Brody et al. (2006; 2004) was not stated. Several other studies described correlations between smoking or nicotine-induced  $\Delta BP_{ND}$  and the hedonic/mood-altering effects of smoking. All these reported positive correlations between  $\Delta BP_{ND}$  and change in euphoria (Barrett, 2004) or change in mood from "sad" to "happy" (Brody, 2009; Montgomery, 2007). However, all relationships were bidirectional, involving both increases and decreases in DA, which complicates interpretation. In this manner, increased DA is associated with increased euphoria or more happiness, but at the same time, decreased DA is also associated with decreased euphoria or more sadness. While this implies that increases and decreases in DA act in an exactly opposing manner, direct evidence to corroborate such a binary role for changes in DA is lacking. Further complicating the issue, none of the above relationships are purely bidirectional across all subjects. For example, in the association reported by Brody et al. (2009), striatal DA release was related to improved mood in some subjects, and reduced DA was related to decreased mood in others. However, in other subjects, DA release was associated with decreased mood, while reduced DA was associated with increased mood in others still. Though it is tempting to speculate on causal relationships between DA release and subjective mood, interpretations of bidirectional associations, such as those cited above, should be made carefully.

Studies of THC-induced DA release in humans have also yielded equivocal results. Two studies that used oral (Stokes, 2009) and IV THC (Barkus, 2011b),

reported no significant striatal DA elevations, although subjects in both studies experienced symptoms typical of THC intoxication. In contrast, a study by Bossong et al. (2009) found that inhaled THC vapor elicited a small but significant DA release in the VST and preDPU. Again, as the vapor inhalation paradigm more closely mimics natural intake of the drug, the DA release reported by this study may have been related to conditioned cues of drug intake (to be discussed in a later section). In addition, a recent investigation used a similar inhalation method in three groups of cannabis users: subjects with low risk for psychosis (controls), subjects with diagnosed psychosis (patients), and subjects with a first-degree relative with diagnosed psychosis (relatives) (Kuepper, 2013). They documented DA release in the dorsal striatum of both patients and relatives, but not controls. Interestingly, the magnitude of DA release was not associated with the behavioral response to THC in any group. No correlations between THC-induced DA release and subjective responses to intoxication were detected in any of these investigations.

Studies of opiate-induced DA release in abusers have produced more concordant results. Two studies in heroin addicts reported neither significant opiate-induced changes in striatal DA, nor correlations of DA release to subjective ratings of high (Daglish, 2008; Watson, 2013).

Taken together, results from the above studies have answered a great deal of questions about how human DA systems respond to acute drug administration, yet many questions remain unanswered. While there is little doubt about the importance of DA in addiction, the specific roles played by DA in mediating drug effects are unclear. A number of studies reported correlations between drug-induced striatal DA release and subjective effects of drug, but the issue is complicated by studies that found no such relationships. Heterogeneity among subject populations, including differences in gender, genotype, severity of addiction and use status (currently using or detoxified), and varying sample sizes may all mediate these discrepant results. One commonality running through all these studies is that the mode of drug administration seemingly has large effects on the study outcome. Specifically, studies that utilized a mode of administration similar to the natural route of intake demonstrate a trend towards greater DA release than those studies attempting to investigate a purely pharmacological effect of drug. The next section will explore the DA response to conditioned drug cues.

### *DA Response to Conditioned Drug Cues in Humans*

There is indirect evidence from several of the above studies that DA may mediate the response to drug conditioned cues. The fact that greater DA release was detected in studies using a more natural route of drug administration (e.g. oral consumption of alcohol vs. IV administration, inhalation of THC vs. oral consumption of a tablet) suggests that conditioned cues that have been repeatedly paired with drug intake (e.g. smell, taste) may activate the striatal DA system separately from than the pharmacological effects alone. In line with this, Domino et al. (2013) showed that smoking a denicotinized cigarette resulted in dorsal striatal DA release compared to a pre-smoking baseline. Though these cigarettes did contain a small amount of nicotine (0.08mg), much larger doses were unable to elicit detectable amounts of DA in other studies (Montgomery, 2007; Tsukada, 2002). Furthermore, several other investigations have attempted to provoke striatal DA release using only cues specifically associated with certain drugs of abuse.

Three separate studies in heavy cocaine abusers compared RAC or FAL BP<sub>ND</sub> between two scans: one during presentation of neutral cues, and the other during presentation of cocaine cues (Fotros, 2013; Volkow, 2006b; Wong, 2006). In each of these, striatal DA was significantly elevated during cocaine cue presentation, but regional release differed slightly. Fotros et al. (2013) reported DA release throughout the whole striatum, but DA release was confined to the dorsal striatum in the other studies. Interestingly, in the studies by Fotros et al. (2013) and Wong et al. (2006), subject responses to the cocaine cues were highly variable, such that the investigators divided them into subgroups of “high cravers”, who reported increased craving during cocaine cues, and “low cravers”, who reported decreased or negligible craving during cocaine cues. After this separation, only “high cravers” exhibited significant DA release during cocaine cues relative to neutral cues in both studies. Furthermore, each study reported that cue-induced striatal DA release was positively correlated with craving, such that greater DA release was associated with a more positive cue-induced change in craving. However, all subjects were included in these correlations in two studies (Fotros, 2013; Volkow, 2006b), while only the “high cravers” were included in the other (Wong, 2006). Whether or not the correlations would have survived if “high” or “low” cravers were analyzed separately was not indicated in the former two studies. Though a relationship between DA release and craving is supported by the general agreement in these studies, the correlations were not consistent across samples, such that the

association of increased DA with reduced craving was present in only a subset of subjects in each study.

A similar study conducted in heroin addicts reported significantly greater cue-induced DA release in the putamen of addicts compared to healthy controls (Zijlstra, 2008). They also reported an inverse correlation between cue-induced craving and baseline  $BP_{ND}$  in the putamen, but not with  $\Delta BP_{ND}$ . It is important to note that they only compared relative  $\Delta BP_{ND}$  between groups, and it is unclear whether there was significant cue-induced DA release in addicts alone. Indeed, in the region where the authors reported a significant group effect on  $\Delta BP_{ND}$ ,  $\Delta BP_{ND}$  was moderately positive (5%) in heroin addicts, and was more strongly negative in controls (-10%). This discrepancy could have artificially inflated the group difference in  $\Delta BP_{ND}$ , and a within-group analysis may not have yielded significant  $\Delta BP_{ND}$  for either group.

Finally, a recent study conducted in a spectrum of alcohol drinkers, whose drinking habits ranged from social to heavy, demonstrated VST DA release in response to beer flavor compared to a Gatorade control flavor (Oberlin, 2013). This effect of beer flavor on DA release was mediated by family history of alcoholism; when the subjects were separated based on degree of family history, significant DA release was detected only in subjects with a first degree alcoholic relative. Although the other family history groups displayed increased DA, the release was not significant.

Taken together, these results indicate that the striatal DA system in humans is responsive to conditioned cues associated with drugs of abuse. Interestingly, cue-induced DA release was confined to more dorsal striatal areas in heavily addicted subjects, whereas it was mainly ventral in subjects with a less severe degree of addiction. This difference could be due to differences in modality of cue presentation, but it is also possible that processing of drug-related stimuli shifted to more dorsal aspects of the striatum after years of abuse (see above section on temporal changes in DA signaling). In support of this, a recent fMRI study showed that alcohol-related visual cues activated ventral striatal areas in light drinkers, but only dorsal striatal regions in heavy drinkers (Vollstadt-Klein, 2010). Additionally, results from the above studies emphasize the importance of careful study design and analysis when examining DA release in response to drugs of abuse.

### *Addiction and Impulsivity – Specific Contributions of DA*

Addiction is a disorder marked by loss of control over substance intake and by impulsive behavior. Individuals suffering from addiction display more impulsive behavior on a wide array of neuropsychological indices of impulsivity (for a review, see De Wit, 2009). Additionally, a large body of research posits the DA system as an important modulator of impulsive behavior, as many brain regions implicated in impulsivity are under control of DAergic transmission (see Chapter 3 for a discussion of the neurophysiology of impulsivity). PET imaging offers a unique opportunity to compare baseline or activated DA state to different types of impulsive phenotypes. In humans, impulsivity is measured a number of different ways, including self-report (e.g. personality questionnaires, history of impulsive behavior), or via performance on cognitive tasks (e.g. stop-signal task, delay-discounting). Several imaging studies have examined relationships between estimates of D<sub>2</sub> receptor availability/DA release with measures of impulsivity. Two RAC studies in healthy controls reported that scores on the non-planning impulsivity subscale of the Barratt Impulsivity Scale (BIS-11, Patton, 1995) were positively correlated with baseline BP<sub>ND</sub> in the preDCA (Kim, 2013) and ventral striatum (Reeves, 2012). Higher BIS-11 scores indicate greater impulsivity, so these results suggest that higher D<sub>2</sub> receptor availability is related to higher impulsivity. Conversely, another study reported a significant negative correlation between baseline FAL BP<sub>ND</sub> and total BIS-11 score in methamphetamine addicts in all striatal regions, though strongest in the DCA (Lee, 2009). Correlations between BIS-11 scores and FAL BP<sub>ND</sub> in healthy controls were negative, though none reached significance. A positive relationship between amphetamine-induced DA release in the striatum, but not baseline striatal BP<sub>ND</sub>, and BIS-11 score has also been documented in healthy controls (Buckholtz, 2010). Baseline FAL binding in the SN/VTA region in this study was negatively correlated with total BIS-11 score. In agreement with the latter finding, baseline FAL binding in the SN/VTA in controls was negatively correlated with novelty seeking (Zald, 2008), a subscale on the tridimensional personality questionnaire (TPQ; Cloninger, 1987). Similar to the BIS-11, higher scores on the TPQ signify greater impulsivity; thus, these results indicate that greater numbers of D<sub>2</sub> autoreceptors in the SN/VTA may be associated with relatively less impulsiveness [although an opposite effect was detected with PHNO in both pathological gamblers and controls (Boileau, 2013)]. Other studies have reported positive relationships between novelty seeking and baseline striatal D<sub>2</sub> availability (Huang, 2010), as well as amphetamine-induced DA

release in the VST (Leyton, 2002). Interestingly, the seemingly opposing relationships between baseline  $D_2$  availability and impulsive measures in these studies could potentially be explained by a U-shaped association between  $D_2$  receptor availability and some impulsive constructs. Indeed, some recent empirical evidence does support this hypothesis (Clark, 2012; Gjedde, 2010), but further work is needed. Though many studies have used self-reported measures of impulsivity in their comparison with DAergic markers, only one study to date has investigated the relationship between functional measures of impulsivity (as measure by the stop-signal task) and estimates of  $D_2$  availability (Ghahremani, 2012). In the study, FAL  $BP_{ND}$  in the dorsal striatum was negatively correlated with stop signal reaction time (SSRT), indicating that lower  $D_2$  receptors levels, or higher DA concentrations, are associated with higher impulsivity. Furthermore,  $D_2$  availability in the DCA was also positively correlated with task-induced fMRI activation in the DCA and several cortical regions. The combined results of these studies strongly implicate the DA system as a critical modulator of impulsivity, but high variability of results complicates the interpretation.

The above sections provide a critical review of studies employing dopaminergic PET to investigate addiction phenotypes. There is overwhelming evidence supporting an integral role for DA in substance abuse disorders. Baseline DA receptor availability has been associated with chronic substance use in general, as well as with impulsive personality traits. Changes in DA receptor availability in response to a pharmacological challenge or cue presentation have been instrumental in characterizing the pharmacological effects, or lack thereof, of certain substances. Cue presentation investigations have also yielded novel information about DAergic involvement during the experience of craving. Future studies should be crafted with careful consideration of matching controls, study design, analysis, and interpretation.

## Chapter 1: Baseline striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in chronic cannabis users

This chapter describes the use of [<sup>11</sup>C]raclopride PET to characterize differences in the striatal DA system between currently-using chronic cannabis users and healthy controls. In addition, baseline D<sub>2</sub>/D<sub>3</sub> receptor availability in cannabis users was investigated for correlations with recent use of cannabis, cannabis craving, and personality indices. Eighteen right-handed males age 18-34 were studied. Ten subjects were chronic cannabis users; eight were demographically matched controls. Subjects underwent a [<sup>11</sup>C]raclopride (RAC) PET scan. Striatal RAC binding potential (BP<sub>ND</sub>) was calculated on both region of interest (ROI) and voxel-wise bases. Prior to scanning, urine samples were obtained from cannabis users for quantification of urine Δ-9-tetrahydrocannabinol (THC) and THC metabolites (11-nor-Δ-9-THC-9-carboxylic acid; THC-COOH and 11-hydroxy-THC;OH-THC).

Results from this analysis support previous studies that found no differences in D<sub>2</sub>/D<sub>3</sub> receptor availability between cannabis users and controls. Voxel-wise analyses revealed that RAC BP<sub>ND</sub> values were negatively associated with both urine levels of cannabis metabolites and self-report of recent cannabis consumption. In this sample, current cannabis use was not associated with deficits in striatal D<sub>2</sub>/D<sub>3</sub> receptor availability. There was an inverse relationship between chronic cannabis use and striatal RAC BP<sub>ND</sub>. This article, which was published in *Drug and Alcohol Dependence* in 2012, supports the need for additional studies to identify the neurochemical consequences of chronic cannabis use on the dopamine system.

## Introduction

Marijuana (*Cannabis sativa*) is one of the most commonly abused illicit drugs in the United States. Over 106 million people age 12 and above (42%) have reported using cannabis at least once. Although the addictive liability of cannabis is a source of debate, cannabis dependence remains a serious health concern (Clapper, 2009): over 1,000,000 Americans received treatment for cannabis abuse or dependence within the past year (Samhsa, 2010). The large number of Americans at risk for cannabis abuse and dependence necessitates a better understanding of the neurobiology of cannabis use disorders.

The main psychoactive component of cannabis,  $\Delta^9$ -tetrahydrocannabinol (THC), exerts its effects via binding the cannabinoid type 1 (CB1) receptor (Devane, 1988; Herkenham, 1991; Herkenham, 1990; Mailleux, 1992). CB1 receptors are expressed throughout the brain, with high densities in the cortex, hippocampus, cerebellum, and striatum. This heterogeneous distribution of CB1 has been confirmed in both humans and non-human primates (Egan, 2007). The role of the striatum in cannabis use is of particular interest, as this structure is often involved in multiple cognitive processes that subserve addiction. The striatum is heavily innervated by midbrain dopamine (DA) neurons, and striatal dopaminergic neurotransmission is believed to mediate both the development and maintenance of addictions (for review see Robinson, 2001; Robinson, 2003).

There is a growing body of *in vivo* evidence that suggests striatal DA receptors may be altered in human addicts. PET and SPECT imaging studies have documented deficits in striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in several populations of abstinent and/or detoxified substance-dependent individuals, including users of cocaine (Martinez, 2009; Volkow, 1997), methamphetamine (Volkow, 2001b), opiates (Wang, 1997b), and alcohol [(Hietala, 1994; Martinez, 2005; Volkow, 1996; Volkow, 2002), although see (Guardia, 2000a; Repo, 1999a)]. Interestingly, this phenomenon has not been demonstrated in cannabis users. Three studies investigating striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in subjects with a history of cannabis use found negligible differences between cannabis users and controls (Sevy, 2008a; Stokes, 2011; Urban, 2012a). However, these studies were conducted in subjects that had been abstinent from cannabis for an average of 15 weeks (Sevy, 2008a), 18 months (Stokes, 2011), and 4 weeks (Urban, 2012a). There is evidence to suggest that reduced D<sub>2</sub>/D<sub>3</sub> receptor availability in addicts may recover after



extended periods of abstinence (Volkow, 2002), although the rate of recovery is highly variable between individuals (Nader, 2006).

In order to better understand the role of DA in cannabis dependence, it is crucial to study individuals who are current heavy cannabis users. To date, no one has examined striatal D<sub>2</sub>/D<sub>3</sub> binding in currently-using chronic cannabis users. Here, we used PET and [<sup>11</sup>C]raclopride (RAC), a D<sub>2</sub>/D<sub>3</sub> antagonist, to compare striatal D<sub>2</sub>/D<sub>3</sub> availability in currently-using chronic cannabis users and age-matched healthy controls. We hypothesized that RAC binding availability would be lower in chronic cannabis smokers relative to controls.

## Methods

All study procedures were approved by the Indiana University Institutional Review Board. Subjects were recruited by local advertising in the greater metropolitan Indianapolis area. All subjects signed an informed consent statement. Eighteen right-handed males completed the study. Participants in the cannabis group (CAN; *n* = 10) were chronic cannabis users, defined by consumption of at least one “joint” per week (or equivalent) in the last month and a positive result for THC on a urine toxicology screen (Skosnik, 2008a; Skosnik, 2006; Skosnik, 2008b). Control subjects (CON; *n* = 8) were non-cannabis smoking males with negative urine toxicology screens. Groups were matched for age and race. Subjects underwent a screening interview that included: the Structured Clinical Diagnostic Interview for DSM-IV disorders (SCID) I and II, and the Edinburgh handedness inventory (Oldfield, 1971). Patterns of alcohol and substance use were ascertained using the SCID I module E for Substance Use Disorders. Exclusion criteria were: history of any neurological disorder, current use of medications with CNS effects, consumption of > 14 alcoholic beverages per week, contraindication for magnetic resonance imaging (MRI), use of any illicit substance during the past three months (except cannabis in CAN subjects), positive urine toxicology screen (other than cannabis in CAN subjects), and DSM-IV diagnosis of an Axis I or II psychiatric disorder (other than nicotine abuse or dependence in any subject, and cannabis abuse or dependence in CAN subjects). History of illicit substance abuse or dependence (other than cannabis in CAN) was exclusionary for all subjects.

### *General Study Procedures*

On a day subsequent to the screening visit, qualified subjects received a structural magnetic resonance image (MRI) and one [ $^{11}\text{C}$ ]raclopride PET scan. Before scanning, subjects reported recent substance use-patterns using an internally developed drug-use questionnaire. All subjects submitted a urine sample for drug toxicology screening. Urine toxicology screens (Q10-1, Proxam) were administered prior to scanning to corroborate self-report and clinical interview. For quantitative cannabinoid analysis, urine samples from CAN subjects were submitted to The Center for Human Toxicology at the University of Utah for quantification of  $\Delta^9$ -tetrahydrocannabinol (THC), 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THC-COOH), 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (OH-THC), and creatinine. CAN subjects were instructed to refrain from smoking cannabis the morning before the scan to help ensure they would not be intoxicated at the time of scanning.

### *Image Acquisition*

A magnetized prepared rapid gradient echo (MP-RAGE) MRI was acquired on all subjects using a Siemens 3T Trio for anatomic co-registration of PET data. RAC was synthesized as reported previously (Fei, 2004a). RAC PET scans were acquired on an ECAT HR+ (3D mode; septa retracted). Prior to each PET scan, a 10-min transmission scan using three internal rod sources was acquired for attenuation correction. RAC PET scans were initiated with an IV infusion of  $544.39 \pm 38.7$  MBq RAC over the course of 1.5 minutes. Injected mass was  $0.17 \pm 0.08$  nmol/kg. Dynamic data acquisition lasted 50 minutes.

During scanning, CAN subjects responded to statements designed to assess cannabis craving. These included: "I want to smoke cannabis right now"; "I have an urge to smoke cannabis right now"; "It would be great to use cannabis right now"; "Nothing would be better than smoking cannabis right now." Responses were given on a Likert-like scale, anchored by 1 (strongly disagree) and 7 (strongly agree). The area under the curve (AUC) for responses to each of the cannabis craving statements was calculated using the trapezoidal rule. The average AUC value across all 4 statements was used as an overall craving metric.

### *Image Processing*

Image processing is similar to that described previously (Yoder, 2011a; Yoder, 2012a). MRI DICOM and RAC PET images were converted to Neuroimaging Informatics Technology Initiative (NIfTI) format (<http://nifti.nimh.nih.gov/>) and processed with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/>). For each subject, an early-time mean PET image was co-registered to the MRI scan using the normalized mutual information algorithm in SPM5. All dynamic PET data were co-registered to the early-time mean PET image (in native MR space) to facilitate motion correction. Each subject's MRI was spatially normalized to Montreal Neurological Institute (MNI) space, and this transformation matrix was then applied to the motion-corrected, MRI-registered PET data from each subject.

### *Region of Interest Analysis*

Regions of interest (ROIs) were drawn on each subjects' normalized MRI using MRIcron (<http://www.cabiatl.com/mricro/mricron/>). Striatal ROIs consisted of the left and right ventral striatum (VST), pre- and postcommissural dorsal caudate (pre-/post-DCA), and pre- and postcommissural dorsal putamen (pre/post-DPU) and were drawn according to specific anatomic landmarks (Martinez, 2003). For the reference region (tissue that contains little to no D<sub>2</sub>/D<sub>3</sub> receptor density), an ROI was created that contained all cerebellar gray matter except for the vermis. Cerebellar ROIs were created for each subject by tracing the cerebellum on individual gray matter maps obtained with the segmentation algorithm in SPM5. Time-activity curves for all ROIs were generated from the dynamic RAC data using the MarsBaR toolbox for SPM5 (<http://marsbar.sourceforge.net/>). For each striatal ROI, D<sub>2</sub>/D<sub>3</sub> receptor availability was indexed with BP<sub>ND</sub>, the binding potential of RAC calculated as bound tracer concentration relative to nondisplaceable tracer concentration (Innis, 2007). Estimations of BP<sub>ND</sub> were conducted using the multilinear reference tissue method model (MRTM2; Ichise, 2003).

### *Voxel-wise Analysis*

BP<sub>ND</sub> was estimated at each brain voxel using the multilinear reference tissue method with a common reference region efflux rate to facilitate robust performance on noisy voxel data (MRTM2; Ichise, 2003). The resulting parametric BP<sub>ND</sub> images were smoothed with an 8mm Gaussian kernel (Costes, 2005; Picard, 2006; Ziolko, 2006).

The search area for the voxel-wise paired *t*-tests was restricted to the striatum, as (1) our sole focus was the striatum, and (2) the striatum has the highest density of D<sub>2</sub>/D<sub>3</sub> receptors in the brain, and is the only brain structure with high enough signal-to-noise ratio to support quantification of D<sub>2</sub>/D<sub>3</sub> receptor availability with RAC. A bilateral striatal restriction mask was created by tracing the anatomical boundaries of the striatum on an averaged normalized MRI across all subjects.

### *Urinalysis*

**THC and THC-COOH:** The samples were initially analyzed for THC and THC-COOH by gas chromatography-mass spectrometry (GC-MS) using extraction and GC-MS conditions described previously (Foltz, 1983; Huang, 2001). The assay had an analytical range of 0.5 to 100 ng/mL with 1.0 mL aliquots. To ensure measurement of both analytes, the urine samples were analyzed for THC on a 1.0-mL aliquot and THC-COOH on a 0.1-mL aliquot. For THC, the aliquots were pretreated with  $\beta$ -glucuronidase for 18 hours at 37°C. For THC-COOH, the samples were prepared under basic conditions in order to free THC-COOH from its glucuronide conjugate. Duplicate calibrators (1.0 mL with both THC-COOH and THC) were at 0.5, 1.0, 2.5, 5, 10, 25, 50 and 100 ng/mL. Duplicate 1.0-mL (with both THC-COOH and THC) quality control samples (QCs) were included at 1.5, 10 and 80 ng/mL. Triplicate 0.1 mL dilution QCs were included at 200 ng/mL. Samples were extracted by a liquid-liquid procedure, derivatized with hexafluoroisopropanol/trifluoroacetic anhydride, and analyzed by GC-MS.

Subsequently, the method was improved by using gas chromatography-tandem mass spectrometry (GC-MS/MS) with addition of 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (OH-THC) to the assay. This assay had a quantitative range of 0.1 to 100 ng/mL with a 1.0-mL aliquot. All samples were reanalyzed to determine OH-THC with the  $\beta$ -glucuronidase pretreatment using the above methods. Samples with THC or THC-COOH results less than the lower limit of quantitation in the initial analysis were reanalyzed.

**Creatinine** – Creatinine was determined using a microplate colorimetric test based on the Jaffe reaction where picric acid reacts with creatinine to form a colored product. Samples were diluted 10-fold (0.050 mL plus 0.450 mL water). Duplicate creatinine calibrators were run at 2, 4, 6, 8, 10, 12 and 15 mg/dL. Due to sample dilution, the calibration range was 20 to 150 mg/dL. Triplicate diluted low and high QCs

were included. Samples outside the calibration range were repeated using a smaller or larger dilution as needed. THC, THC-COOH, and OH-THC concentrations were normalized by creatinine levels to account for differing levels of urine dilution across subjects (THC/Cr, THC-COOH/Cr, and OH-THC/Cr respectively).

### *Statistical Analysis*

Independent *t*-tests were used to test for differences between CAN and CON in demographic variables, substance abuse metrics, PAS, and SPQ scores, and RAC BP<sub>ND</sub>. Group differences in BP<sub>ND</sub> were assessed with ROI and voxel-wise analyses. Pearson's correlation coefficient was used to screen variables for associations with striatal ROI BP<sub>ND</sub>. Multiple linear regression models in SPM5 were used to test for correlations on a voxel-wise basis. SPM5 was used for voxel-wise analysis, statistical threshold was set at  $p < 0.05$ . All other statistical procedures were performed in SPSS 19.0 (SPSS, Chicago, Illinois, USA).

## Results

### *Subject Data*

The demographic and substance abuse characteristics of subjects are shown in Table 1. CAN and CON subjects were not significantly different in any of the indices. Groups were well-matched for race, ethnicity, and use of alcohol, tobacco, and caffeine. There were no significant differences between injected radioactivity or injected mass between groups ( $p > 0.1$ ).

### *Urine THC and THC Metabolite Corroborate Self-Report of Cannabis Consumption*

THC/Cr, THC-COOH/Cr, and OH-THC/Cr levels were correlated with self-reported recent cannabis use. One subject was excluded from this analysis because of inconsistent self-report data. Significant positive correlations existed between: intake per day and THC-COOH/Cr ( $r = 0.884$ ,  $p = 0.002$ ), intake per day and THC/Cr ( $r = 0.738$ ,  $p = 0.023$ ), intake per week and THC-COOH/Cr ( $r = 0.726$ ,  $p = 0.027$ ), and intake per month and THC-COOH/Cr ( $r = 0.676$ ,  $p = 0.045$ ). There was a trend-level association between intake per day and OH-THC/Cr ( $r = 0.647$ ,  $p = 0.059$ ). There were no significant correlations between THC/Cr, THC-COOH/Cr, or OH-THC/Cr and cannabis craving during PET scanning.

**Table 1.** Subject demographics and drug-use characteristics. THC use is defined as a one-time session of THC intoxication. Data are mean  $\pm$  s.d. Healthy controls: CON; currently using chronic cannabis users: CAN; Caucasian: C; African American: AA; Asian-Indian American: I; Non-Hispanic Latino: NHL; not applicable: N/A. Recent EtOH use is average drinks per week in the past month.

	CON ( <i>n</i> = 8)	CAN ( <i>n</i> = 10)	<i>p</i>
Age	26.4 $\pm$ 5.6	25.1 $\pm$ 4.6	n.s.
Race	7C, 1AA	6C, 3AA, 1I	n.s.
Ethnicity	8 NHL	10 NHL	n.s.
Education	14.6 $\pm$ 1.3	14.0 $\pm$ 1.8	n.s.
Recent THC use/wk	N/A	12.7 $\pm$ 12	
Recent THC use/month	N/A	46.6 $\pm$ 42	
Years of THC use	N/A	8.8 $\pm$ 5	
Hours since last THC use	N/A	20.6 $\pm$ 8.3	
Tobacco users	2	5	n.s.
Caffeine users	5	6	n.s.
Recent EtOH use	2.94 $\pm$ 2.0	3.93 $\pm$ 3.7	n.s.
Premorbid IQ	112.3 $\pm$ 6.9	110.2 $\pm$ 4.4	n.s.
Prior Drug Use: (lifetime drug use sessions)			
THC	36.1 $\pm$ 71.7	2571.4 $\pm$ 2490.5	0.01
Sedatives	0	0.65 $\pm$ 1.7	n.s.
MDMA	0	1.20 $\pm$ 3.1	n.s.
Stimulants	0	0.20 $\pm$ 0.4	n.s.
Cocaine	0.63 $\pm$ 1.8	4.20 $\pm$ 6.3	n.s.
Opiates	0	0.10 $\pm$ 0.3	n.s.
Hallucinogens	1.31 $\pm$ 2.7	1.20 $\pm$ 1.6	n.s.

### *Striatal D<sub>2</sub>/D<sub>3</sub> Availability*

#### CAN vs. CON

There were no significant between group differences in RAC BP<sub>ND</sub> detected by voxel-wise analysis. Similarly, no group differences were found for any of the 10 striatal ROIs assessed ( $p > 0.4$ ) (Table 2).

#### Correlation with Recent Cannabis Consumption

Voxel-wise analysis revealed that RAC BP<sub>ND</sub> was negatively associated with both urine levels of THC-COOH (Figure 1) and self-reported recent intake per day (Figure 2). Similar correlations were found between BP<sub>ND</sub> and THC/Cr, OH-THC/Cr, recent intake per week, and recent intake per month (data not shown).

### Discussion

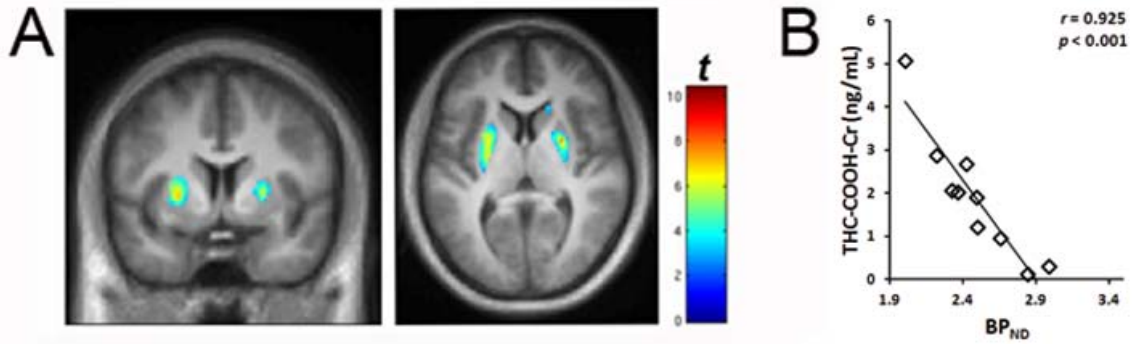
The present work is the first to demonstrate an association between the magnitude of recent cannabis consumption and striatal D<sub>2</sub>/D<sub>3</sub> receptor availability. RAC BP<sub>ND</sub> was strongly negatively correlated with both urine THC-COOH and self-reported recent intake per day. We did not find the expected differences in striatal D<sub>2</sub>/D<sub>3</sub> receptor availability between cannabis users and controls, similar to what has been reported previously (Sevy, 2008a; Stokes, 2011; Urban, 2012a).

The inverse correlation between recent cannabis consumption (as confirmed by urine THC metabolite levels) and D<sub>2</sub>/D<sub>3</sub> receptor availability could be interpreted as a direct effect of cannabis smoking via lower expression of striatal DA receptors, or increased basal DA concentration. There is evidence that suggests that heavy cannabis use results in inhibition of MAO activity (Schurr, 1984; Stillman, 1978), and thus a higher striatal DA tone (Kaseda, 1999; Lakshmana, 1998; Lamensdorf, 1996). Alternatively, activation of CB1 receptors may also result in higher striatal DA concentration (Chen, 1990; Fadda, 2006b; Tanda, 1997b). We must consider the possibility that, in this study, residual THC from the most recent smoking session increased striatal DA levels; however, several lines of evidence suggest otherwise. Human imaging studies have attempted to demonstrate THC-induced DA release, with inconclusive results. One study reported a small (3%) increase in striatal DA after inhaled THC (Bossong, 2009), while two other groups detected no increases in striatal DA after either oral (Stokes, 2009) or IV-delivered THC (Barkus, 2011a). Additionally, in the present work, it is

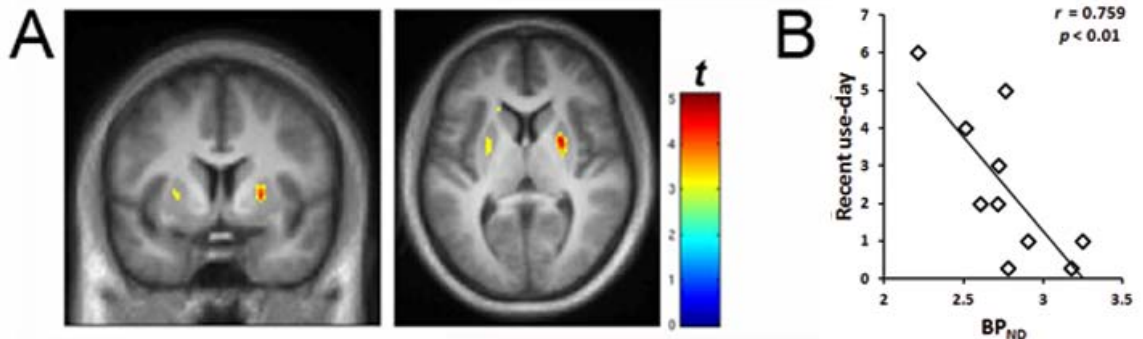
**Table 2.** Region of interest analysis: comparison of striatal binding potential between chronic cannabis users (CAN) and healthy controls (CON). Groups are matched for cigarette smoking status. Left/right: L/R; pre/post-commissural: pre/post; dorsal caudate: DCA; dorsal putamen: DPU; ventral striatum: VST.

<b>[<sup>11</sup>C]RACLOPRIDE RECEPTOR AVAILABILITY (BP<sub>ND</sub>)</b>			
<b>Region</b>	<b>CON (n = 8)</b>	<b>CAN (n = 10)</b>	<b>p</b>
L pre-DCA	2.37 ± 0.29	2.30 ± 0.32	0.63
R pre-DCA	2.34 ± 0.30	2.23 ± 0.29	0.44
L post-DCA	1.51 ± 0.27	1.59 ± 0.28	0.56
R post-DCA	1.55 ± 0.33	1.64 ± 0.19	0.52
L pre-DPU	3.08 ± 0.32	2.92 ± 0.29	0.28
R pre-DPU	3.04 ± 0.30	2.94 ± 0.22	0.44
L post-DPU	3.11 ± 0.31	2.98 ± 0.32	0.40
R post-DPU	3.00 ± 0.33	2.92 ± 0.32	0.63
L VST	2.52 ± 0.29	2.47 ± 0.33	0.78
R VST	2.29 ± 0.25	2.35 ± 0.23	0.58





**Figure 1. A.** Voxel-wise correlations between urine THC-COOH/Cr with RAC BP<sub>ND</sub> in cannabis users ( $n = 10$ ). The “rainbow” colorscale indicates voxels where BP<sub>ND</sub> is correlated with THC-COOH/Cr. **B.** Linear relationship between BP<sub>ND</sub> and urine THC-COOH levels. Average BP<sub>ND</sub> value was determined for each subject by extracting BP<sub>ND</sub> values with a region of interest defined by the significant voxels from the SPM result (shown in 1A). Display threshold is  $p < 0.01$ . MNI coordinates are: axial: 6; coronal: 24.



**Figure 2. A.** Voxel-wise correlations between self-reported average intake per day and RAC BP<sub>ND</sub> in cannabis users ( $n = 9$ ). The “rainbow” colorscale indicates voxels where BP<sub>ND</sub> is correlated with average use per day. **B.** Linear relationship between BP<sub>ND</sub> values and recent cannabis use per day. Average BP<sub>ND</sub> value was determined for each subject by extracting BP<sub>ND</sub> values with a region of interest defined by the significant voxels from the SPM result (shown in 2A). Display threshold is  $p < 0.01$ . MNI coordinates are: axial: 6; coronal: 24.

unlikely that brain levels of THC or psychoactive metabolite were sufficient to induce measurable DA release. In a recently described pig model that closely mimics the kinetic profile of THC in humans, the concentration of a dose of IV-administered THC was greatly reduced in the brain after six hours, and completely absent after 24 hours (Brunet, 2006). Given that subjects in the present study had abstained from smoking an average of 20.6 hours prior to scanning, it is likely that brain levels of THC were negligible.

It is also possible that the relationship between cannabis consumption and striatal D<sub>2</sub>/D<sub>3</sub> receptor availability is a result of lower D<sub>2</sub>/D<sub>3</sub> receptor numbers in heavy cannabis users. Interestingly, evidence from studies of CB1 receptors supports this interpretation. CB1 receptors are co-localized with D<sub>2</sub> receptors in the striatum (Hermann, 2002; Mailleux, 1992; Pickel, 2006; Wenger, 2003) and D<sub>2</sub> receptors and CB1 receptors form heterodimeric receptor complexes (Kearn, 2005). A postmortem study showed that long-term cannabis users possess a marked reduction in the density of CB1 in human brain (Villares, 2007). Additionally, it has been demonstrated that chronic cannabis users exhibit motor learning deficits similar to those observed in CB1 knockout mice, suggesting that long-term cannabis exposure induces robust downregulation and/or desensitization of CB1 receptors (Skosnik, 2008a). This has recently been shown in vivo in humans using the CB1 tracer [<sup>18</sup>F]FMPEP-d2. Hirvonen *et al.* (2011) demonstrated CB1 downregulation in chronic cannabis users, which correlated with total years of cannabis exposure. CB1 availability returned to normal levels after four weeks of monitored abstinence. Taken together, the data from the literature indirectly suggest that chronic exposure to cannabis may lead to downregulation of striatal D<sub>2</sub> receptors. However, in the present study, we did not find differences in D<sub>2</sub>/D<sub>3</sub> receptor availability between controls and chronic cannabis users, suggesting that chronic cannabis exposure alone is not associated with reduced D<sub>2</sub> receptor levels.

Finally, there is one additional putative explanation for the association of recent cannabis consumption and D<sub>2</sub>/D<sub>3</sub> receptor availability. It is possible that individuals with relatively lower D<sub>2</sub>/D<sub>3</sub> receptor availability are predisposed to engage in higher levels of substance use. It has been suggested that lower baseline striatal DA receptor availability is associated with a more positive subjective response to a reinforcing DAergic stimulus (Volkow, 1999), indicating that lower DA receptor availability could confer an increased likelihood to abuse substances. In agreement with this, others have

argued that lower levels of D<sub>2</sub> receptors increase the probability of addictive behavior (Blum, 2000; Blum, 1996), and that higher levels of D<sub>2</sub> receptors might serve as a protective mechanism that reduces the likelihood of substance abuse (Volkow, 2006a). In the current dataset, cannabis users with the highest recent cannabis consumption exhibited the lowest D<sub>2</sub>/D<sub>3</sub> receptor availability. According to the above studies, the predilection to engage in heavier use of cannabis could be a result of relatively lower premorbid levels of D<sub>2</sub> receptors in these subjects. However, if the probability of engaging in substance abuse was indeed associated with lower levels of D<sub>2</sub> receptor expression in the current sample, one would expect to detect significant group differences in D<sub>2</sub>/D<sub>3</sub> receptor availability between cannabis users and controls, which was not the case. This issue is further complicated due to: 1) the cross-sectional nature of the study, as there is no way to resolve differences in DA function that occur prior to substance use from those that are a result of prolonged substance use, and 2) the nature of PET methodology is such that relative contributions of receptor expression levels versus concentration of endogenous DA to BP<sub>ND</sub> cannot be parsed. Longitudinal studies that employ other techniques, such as DA challenges, will be useful in elucidating this issue.

The present study has several limitations. The sample size is relatively small, and thus presents a risk of both Type I and Type II errors. However, our data are consistent with those from Sevy *et al.* (2008a), Stokes *et al.* (2011), and Urban *et al.* (2012a), which reported that striatal D<sub>2</sub>/D<sub>3</sub> receptor availability is not different in individuals with a history of cannabis abuse compared to controls. Finally, although use of any illicit substance within the last three months prior to scanning was an exclusion criterion, both cannabis users and controls had previous experiences with other drugs. Thus, we cannot preclude the possibility that prior use of other illicit substances confounded our data. However, qualitative examination of the data did not indicate that subjects with previous drug experience were outliers with respect to BP<sub>ND</sub>.

In conclusion, the primary finding of the current study is that current cannabis use is not associated with a reduction in striatal DA receptor availability relative to controls. We also found that recent cannabis use is negatively correlated with striatal D<sub>2</sub>/D<sub>3</sub> availability. Future studies are needed to better understand the neurochemical basis of this finding.

## **Chapter 2: Effects of cigarette smoking on striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in alcoholics and social drinkers**

This chapter describes the use of [<sup>11</sup>C]raclopride PET to assess the degree to which comorbid alcohol and tobacco abuse is associated with deficits in the striatal DA system. Eighty-one subjects (34 nontreatment-seeking alcoholic smokers [NTS-S], 21 social-drinking smokers [SD-S], and 26 social-drinking non-smokers [SD-NS]) received baseline [<sup>11</sup>C]raclopride scans. All but seven of the smoking subjects received a transdermal nicotine patch during the scan day. D<sub>2</sub>/D<sub>3</sub> binding potential ( $BP_{ND} \equiv B_{avail}/K_D$ ) was estimated for ten anatomically defined striatal regions of interest (ROIs). ANOVA was used to detect  $BP_{ND}$  differences between the three groups. Pearson's correlation coefficient was used to assess associations between striatal  $BP_{ND}$  and subjective variables.

Results from an ANOVA demonstrated significant group effects in bilateral pre-commissural dorsal putamen, bilateral pre-commissural dorsal caudate, and bilateral post-commissural dorsal putamen. Post-hoc testing revealed that, regardless of drinking status, smokers had lower striatal D<sub>2</sub>/D<sub>3</sub> receptor availability than non-smoking controls. This effect appears to be independent of nicotine patch administration. We hypothesize that the observed effect is related to inhibition of brain monoamine oxidase (MAO) by tobacco combustion products, and subsequently higher intrasynaptic DA concentration. Additional studies are needed to identify the mechanisms by which chronic tobacco smoking is associated with striatal dopamine receptor availability.

## Introduction

Alcohol and tobacco are the two most commonly abused substances in the United States. In people over the age of 12, the percentage reporting lifetime use of alcohol is 82.5%, and 67.5% for tobacco (Samhsa, 2011). These two drugs interact in several domains. Specifically, tobacco cigarette-dependent individuals are approximately six times more likely to be alcohol dependent than non-tobacco cigarette-dependent individuals (Grant, 2004), and alcohol-dependent individuals are over five times more likely to be tobacco cigarette-dependent than non-alcohol-dependent individuals (Hasin, 2007). Comorbid abuse of alcohol and cigarettes has also been associated with higher rates of certain types of cancer than the abuse of either substance in isolation, including oral, laryngeal, esophageal, and liver cancer (Pelucchi, 2006). Evidence for additive effects of alcohol and cigarettes on cardiovascular disease is less conclusive (Mukamal, 2006). Even so, abuse of either substance imparts increased risk for cardiovascular disease. Overall, the economic burden of alcohol and cigarette abuse in the U.S. is estimated at \$185 billion (Harwood, 2000) and \$138 billion (Rice, 1999), respectively.

Many factors likely contribute to the prevalence of comorbid alcohol and cigarette abuse (Drobes, 2002), including the potential overlap of neurobiological mechanisms that subserves alcohol and cigarette dependence. One circuit implicated in most, if not all, addictive processes is the striatal dopamine (DA) system (Di Chiara, 1988a; Koob, 1992; Leshner, 1999; Robinson, 2003; Volkow, 2009). A growing body of *in vivo* evidence suggests that striatal DA receptors may be altered in human addicts. PET and SPECT imaging studies have documented lower striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in several populations of abstinent and/or detoxified substance-dependent individuals, including users of cocaine (Martinez, 2009; Volkow, 1997), methamphetamine (Volkow, 2001b), opiates (Wang, 1997b), alcohol [(Heinz, 2004; Hietala, 1994; Martinez, 2005; Volkow, 1996; Volkow, 2002), although see (Guardia, 2000a; Repo, 1999a)], and cigarette-smoking subjects [(Busto, 2009; Fehr, 2008), although see (Yang, 2006)].

The high occurrence of comorbid alcohol and cigarette abuse, coupled with the association of both substances with deficits in the striatal dopaminergic (DAergic) system, are suggestive that alcohol and cigarettes act similarly on neurobiological circuits that underlie addiction. However, it is currently unknown if the individuals who abuse both alcohol and cigarettes have similar or greater deficits in D<sub>2</sub>/D<sub>3</sub> availability

compared to those who abuse only one substance. To begin to address this question, we conducted a retrospective analysis of baseline [ $^{11}\text{C}$ ]raclopride (RAC) PET data collected from several studies in the laboratory (RAC is a dopamine  $D_2/D_3$  receptor antagonist used to estimate *in vivo* striatal receptor density). Baseline RAC PET data were compiled for three groups: nontreatment-seeking alcoholic smokers (NTS-S), social-drinking smokers (SD-S), and social-drinking non-smokers (SD-NS). We hypothesized that chronic, comorbid alcohol and cigarette abuse would be associated with greater deficits in  $D_2/D_3$  receptor availability than abuse of cigarettes alone.

## Methods

All study procedures were approved by the Indiana University Institutional Review Board and performed in accordance with the ethical standards of the Belmont Report. Subjects were recruited by local advertising in the greater Indianapolis area. After a complete description of the study to the subjects, written informed consent was obtained. Eighty-one right-hand dominant, adult subjects completed study procedures. Data from subsets of subjects have been published previously (Albrecht, 2012b; Yoder, 2011b; Yoder, 2012b). The presence or absence of alcohol abuse or dependence was assessed by either by the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz, 1994) ( $n = 73$ ) or the Structured Clinical Diagnostic Interview for DSM-IV disorders (SCID) I Substance Use Disorders section (Module E) ( $n = 8$ ). For all subjects, the absence of illicit substance abuse or dependence was confirmed by the SCID-I Substance Use Disorders section. Subjects were excluded from participation if they endorsed recreational use of legal or illicit stimulants, pain medications, sedatives, and/or regular consumption of  $>2$  marijuana cigarettes (or equivalent) per week. Urine toxicology screens (Q-10, Proxam) were administered during the screening visit, and on the day of PET imaging. Any positive result for an illicit substance on the screening visit was exclusionary (with the exception of THC when sporadic use was endorsed). Positive results on the day of scanning were recorded. NTS-S subjects had not received treatment for alcohol use disorders within the past year and were not actively seeking treatment. In cigarette smokers, tobacco dependence was assessed with the Fagerström Test for Nicotine Dependence (Pomerleau, 1994); these data were unavailable for three smoking subjects.

### *General Scan Day Procedures*

Subject sobriety was confirmed by BrAC measurement prior to scan day procedures for the majority of subjects ( $n = 73$ ); BrAC was not measured in eight control subjects. Subjects received a structural MRI and a baseline [ $^{11}\text{C}$ ]raclopride (RAC) PET scan. All but seven smoking subjects received a transdermal nicotine patch, which has been shown to effectively control craving; variance in baseline RAC binding is also stable with patch placement (Yoder, 2011b; Yoder, 2012b). Patch placement occurred approximately 5.5 hours before RAC PET scanning (mean, 5.42 hours; range, 1-7 hours). Patch dose was based on subjects' self-report of number of cigarettes smoked per day, per package instructions. Thirty-seven subjects received a 21mg patch, 10 received a 14mg patch, and one subject received a 7mg patch. Cigarette craving was measured with the second dimension of the Cigarette Withdrawal Scale (CWS; (Etter, 2005)), which specifically captures the individual's current subjective state of cigarette craving. There are four items in this dimension, anchored by 1 (totally disagree) and 5 (totally agree). The final metric is a composite sum of the scores for each item; thus, the craving score range is 4-20. Cigarette craving data were available for 47 of the 55 smoking subjects. Forty-two of these subjects completed a paper version of the CWS prior to the rest scan; 5 subjects completed an electronic version immediately after RAC injection. On the day of scanning, two NTS subjects tested positive for cocaine, though both subjects denied recent cocaine use. One NTS and one SD-S subject tested positive for opiates on the scan day; both subjects reported that drugs had been prescribed for recent dental work. As previously described (Yoder, 2011b), NTS subjects were monitored for alcohol withdrawal with the Clinical Withdrawal Assessment for Alcohol, Revised (CIWA-Ar; (Sullivan, 1989)).

### *Image Acquisition*

A magnetized prepared rapid gradient echo (MP-RAGE) magnetic resonance image (MRI) was acquired using a Siemens 3T Trio for anatomic co-registration of PET data. RAC was synthesized as reported previously (Fei, 2004b). RAC PET scans were acquired on an ECAT HR+ (3D mode; septa retracted). Prior to each PET scan, a 10-min transmission scan using three internal rod sources was acquired for attenuation correction. RAC PET scans were initiated with an IV infusion of  $522.4 \pm 55.6$  MBq RAC over the course of 1.5 minutes. Injected mass was  $0.14 \pm 0.07$  nmol/kg. Dynamic data acquisition lasted 50 minutes.



### *Image Processing*

Image processing was similar to that described previously (Yoder, 2011b; Yoder, 2012b). MRI and PET images were converted to Neuroimaging Informatics Technology Initiative (NIfTI) format (<http://nifti.nimh.nih.gov/>) and processed with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/>). For each subject, an early mean PET image was coregistered to the anatomic MRI using the mutual information (MI) algorithm in SPM5. Each subject's MRI was spatially normalized to Montreal Neurological Institute (MNI) space. The transformation matrix obtained from the spatial normalization step was then applied to the motion-corrected, MRI-registered PET data from each subject.

### *Region of Interest Analysis*

Regions of interest (ROIs) were drawn on individual subjects' spatially normalized MRIs using MRIcron (<http://www.cabiatl.com/mricro/mricron/>). Striatal ROIs were drawn according to specific anatomic landmarks (Martinez, 2003; Mawlawi, 2001), and consisted of the left and right ventral striatum (VST), pre- and postcommissural dorsal caudate (pre-/post-DCA), and pre- and postcommissural dorsal putamen (pre/post-DPU). Individual cerebellar ROIs were used as the reference region (tissue that contains little to no D<sub>2</sub>/D<sub>3</sub> receptor density). Individual gray matter cerebellar ROIs were created for each subject; the vermis was excluded. For each subject and each ROI, the number of voxels in the ROI was recorded and converted to volume (cc). Time-activity curves for all ROIs were extracted from the dynamic RAC data using the MarsBaR toolbox (<http://marsbar.sourceforge.net/>). The RAC binding potential for each ROI ((defined as bound tracer concentration relative to nondisplaceable tracer concentration; BP<sub>ND</sub> (Innis, 2007)) was estimated with the multilinear reference tissue method model (MRTM; Ichise, 2003). One subject had a substantial atrophy of the caudate; caudate data from this individual were excluded from analyses.

### *Statistical Analysis*

One-way analysis of variance (ANOVA) was used to test for mean differences in outcome variables across the three groups. To identify sources of significant group effects, *post-hoc* testing was conducted using the Least Square Difference (LSD) method. Bonferroni corrections were applied to account for multiple comparisons. To test for effects of nicotine patch on BP<sub>ND</sub>, one-way ANOVA was conducted in subsets of age-matched smokers, with nicotine patch dose as a fixed factor (no patch; 7/14 mg; 21

mg). Pearson's correlation coefficient was used to test for associations between striatal  $BP_{ND}$  and subjective variables. Statistical tests were performed in Microsoft Excel 2007 or SPSS 19. Unless otherwise specified (e.g., in the case of Bonferroni correction), statistical significance was set at  $p < 0.05$ .

## Results

### *Subject Characteristics*

Subject demographics and drinking characteristics are shown in Table 3. Groups were balanced for handedness, race, ethnicity, and education. Fagerström scores were not significantly different between smoking groups (Table 3). There was a main effect of group for number of drinks per week ( $p < 1.0 \times 10^{-15}$ ). *Post-hoc* testing showed that NTS drank significantly more than both social-drinking groups (Table 3). SD-S and SD-NS did not differ in amount of alcohol consumed per week (Table 3). One-way ANOVA revealed a main effect of age ( $p < 0.05$ ): SD-NS subjects were significantly younger than both SD-S and NTS-S subjects (Table 3). There was a main effect of injected radioactivity ( $p < 0.05$ ). *Post-hoc* testing revealed that injected radioactivity in SD-S subjects was significantly higher than NTS-S subjects (Table 3). Mass dose was not significantly different across the three groups.

### *Striatal $BP_{ND}$ : ROI Analysis*

There was a main effect of group for  $BP_{ND}$  in the L-pre-DCA, L-pre-DPU, L-post-DPU, R-pre-DCA, R-pre-DPU, and R-post-DPU (Table 4). Figure 3 illustrates the distribution of  $BP_{ND}$  in the R-pre-DPU for all three groups. Although only the R-pre-DPU and L-post-DPU survived Bonferroni correction, we observed that the mean  $BP_{ND}$  values for the smoking groups were both lower relative to the non-smokers. To test the hypothesis that  $BP_{ND}$  is lower in the smokers compared to non-smokers, we performed an orthogonal planned contrast within the general linear model framework to compare the mean of the SD-NS group to the combined means of the NTS-S and SD-S groups. Applying Bonferroni correction to account for multiple comparisons lowered the threshold for significance to  $p < 0.005$ . At this corrected significance level, smokers had significantly lower striatal  $BP_{ND}$  values in six regions (Table 5).

**Table 3.** Subject characteristics. Data are mean  $\pm$  s.d. unless otherwise specified. Nontreatment-seeking alcoholic smokers: NTS-S; social-drinking smokers: SD-S; social-drinking non-smokers: SD-NS. CWS: Cigarette Withdrawal Scale.

\* Main effect of group, one-way ANOVA,  $p < 0.05$

† Different from SD-NS, LSD post-hoc test,  $p < 0.05$

‡ Different from NTS-S, LSD post-hoc test,  $p < 0.05$

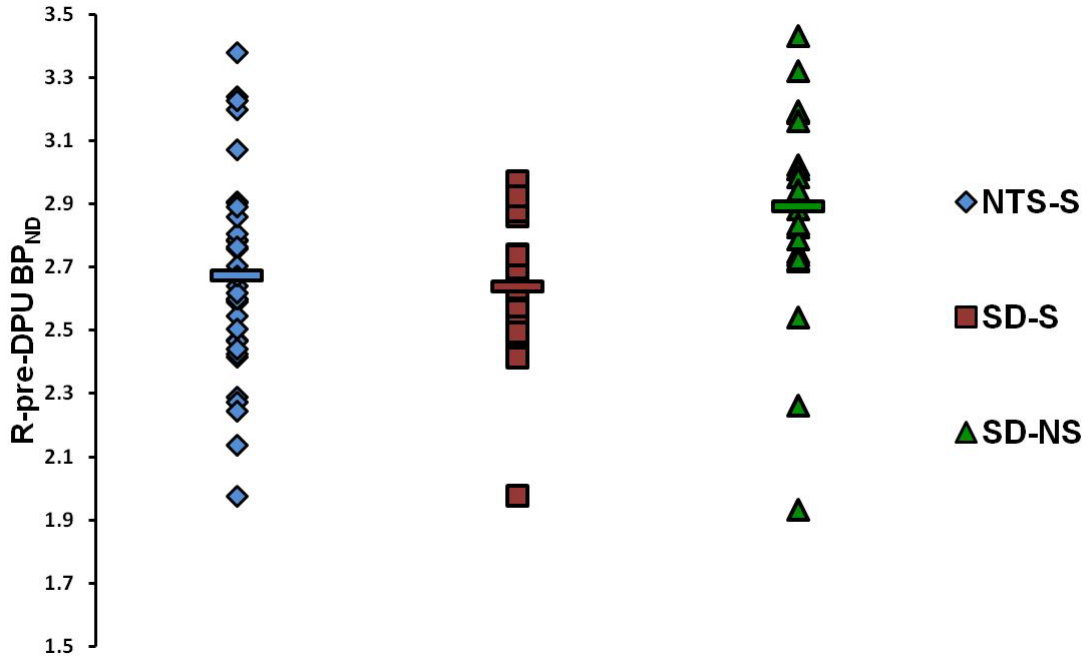
Characteristic	NTS-S (N = 34)	SD-S (N = 21)	SD-NS (N = 26)
Age*	38.4 $\pm$ 8.2 <sup>†</sup>	37.9 $\pm$ 8.7 <sup>†</sup>	30.4 $\pm$ 7.3
Education (years)	12.6 $\pm$ 2.1	13.0 $\pm$ 2.2	14.9 $\pm$ 1.7
Injected radioactivity (mCi)*	13.7 $\pm$ 1.8	14.8 $\pm$ 1.2	14.1 $\pm$ 1.1
Mass dose (nmol/kg)	0.14 $\pm$ 0.06	0.13 $\pm$ 0.05	0.14 $\pm$ 0.09
Fagerström score	4.35 $\pm$ 2.3	4.28 $\pm$ 1.4	N/A
CWS dimension 2	8.53 $\pm$ 4.3	7.82 $\pm$ 4.0	N/A
Drinks/wk*	39.7 $\pm$ 21	4.80 $\pm$ 2.9 <sup>‡</sup>	3.03 $\pm$ 2.6 <sup>‡</sup>
	<b>N (%)</b>	<b>N (%)</b>	<b>N (%)</b>
Caucasian	19 (56)	15 (71)	21 (81)
Male	27 (79)	18 (86)	16 (62)

**Table 4.** Binding potential values ( $BP_{ND}$ ), all groups. Nontreatment-seeking alcoholic smokers: NTS-S; social-drinking smokers: SD-S; social-drinking non-smokers: SD-NS; left/right: L/R; pre/post-commissural: pre/post; dorsal caudate: DCA; dorsal putamen: DPU; ventral striatum: VST.

\* Main effect of group, one-way ANOVA, at  $p < 0.05$ , uncorrected

† Main effect of group with Bonferroni correction ( $p < 0.005$ )

Region	$BP_{ND}$		
	NTS-S (N = 34)	SD-S (N = 21)	SD-NS (N = 26)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
L pre-DCA*	2.11 $\pm$ 0.34	2.13 $\pm$ 0.16	2.29 $\pm$ 0.30
R pre-DCA*	2.11 $\pm$ 0.32	2.05 $\pm$ 0.20	2.27 $\pm$ 0.30
L post-DCA	1.67 $\pm$ 0.34	1.61 $\pm$ 0.20	1.70 $\pm$ 0.30
R post-DCA	1.64 $\pm$ 0.31	1.53 $\pm$ 0.22	1.64 $\pm$ 0.32
L pre-DPU*	2.72 $\pm$ 0.30	2.72 $\pm$ 0.23	2.94 $\pm$ 0.32
R pre-DPU* <sup>†</sup>	2.67 $\pm$ 0.32	2.67 $\pm$ 0.21	2.92 $\pm$ 0.32
L post-DPU* <sup>†</sup>	2.77 $\pm$ 0.30	2.77 $\pm$ 0.24	3.01 $\pm$ 0.33
R post-DPU*	2.68 $\pm$ 0.29	2.71 $\pm$ 0.25	2.95 $\pm$ 0.37
L VST	2.18 $\pm$ 0.31	2.20 $\pm$ 0.26	2.31 $\pm$ 0.30
R VST	2.14 $\pm$ 0.39	2.17 $\pm$ 0.22	2.19 $\pm$ 0.32



**Figure 3.** Individual  $BP_{ND}$  data from the right pre-commissural dorsal putamen (R-pre-DPU), by group. Blue diamonds: nontreatment-seeking alcoholic smokers, NTS-S; red squares: social-drinking smokers, SD-S; green triangles: social-drinking non-smokers, SD-NS. Horizontal lines indicate group means.

**Table 5.** Binding potential values ( $BP_{ND}$ ) from the region of interest (ROI) analysis, stratified by smoking status. For the purpose of presentation, the smoking group data are the mean  $\pm$  S.D. of all subjects in the NTS-S and SD-S groups. Left/right: L/R; pre/post-commissural: pre/post; dorsal caudate: DCA; dorsal putamen: DPU; ventral striatum: VST.

\* Groups significantly different at  $p < 0.05$ , one-way ANOVA with planned contrasts, uncorrected

† Groups significantly different after correction for multiple comparisons ( $p < 0.005$ )

Region	$BP_{ND}$	
	Smoking group (N = 55)	Non-smoking group (N = 26)
	Mean $\pm$ SD	Mean $\pm$ SD
L pre-DCA*	2.08 $\pm$ 0.40	2.29 $\pm$ 0.30
R pre-DCA*	2.08 $\pm$ 0.28	2.27 $\pm$ 0.30
L post-DCA	1.65 $\pm$ 0.29	1.70 $\pm$ 0.30
R post-DCA	1.60 $\pm$ 0.28	1.64 $\pm$ 0.32
L pre-DPU <sup>*,†</sup>	2.72 $\pm$ 0.29	2.94 $\pm$ 0.32
R pre-DPU <sup>*,†</sup>	2.67 $\pm$ 0.28	2.92 $\pm$ 0.32
L post-DPU <sup>*,†</sup>	2.77 $\pm$ 0.27	3.01 $\pm$ 0.33
R post-DPU <sup>*,†</sup>	2.69 $\pm$ 0.27	2.95 $\pm$ 0.37
L VST	2.19 $\pm$ 0.29	2.31 $\pm$ 0.30
R VST	2.15 $\pm$ 0.33	2.19 $\pm$ 0.32

### *Striatal BP<sub>ND</sub>: Effect of Nicotine Patch Dose*

To determine if transdermal nicotine patches have an effect on striatal BP<sub>ND</sub>, we examined data from three age-matched subsets ( $n = 7$  each) of cigarette smokers. This approach is analogous to that recently reported by Weerts et al. (2013) with [<sup>11</sup>C]carfentanil. These subsets included: (1) smokers who did not receive a nicotine patch during the scan day ( $37.6 \pm 7.2$  y.o.), (2) smokers who received a patch dose of 7 or 14mg nicotine (one subject received a 7mg patch, the rest received 14mg;  $38.1 \pm 10.5$  y.o.), and (3) smokers who received a patch dose of 21mg nicotine ( $37.0 \pm 6.4$  y.o.). Groups were also balanced for drinking status: each group contained four SD-S subjects and three NTS-S subjects. One-way ANOVA did not reveal any main effects of patch dose on BP<sub>ND</sub> in any of the ten striatal regions, indicating that there was no effect of nicotine on BP<sub>ND</sub> (data not shown).

### *ROI volumes: Group differences*

There was a main effect of group on ROI volume in the L-pre-DCA, L-pre-DPU, L-post-DCA, R-pre-DCA, R-pre-DPU, and R-post-DCA (Table 6). *Post-hoc* testing revealed that, regardless of smoking status, ROI volumes for both social-drinking groups (SD-S and SD-NS) were significantly greater than those for the NTS-S group in several regions (Table 6).

## Discussion

The current study investigated whether chronic abuse of both alcohol and tobacco cigarettes has a differential effect on D<sub>2</sub>/D<sub>3</sub> receptor availability compared to what has been previously reported for alcohol or tobacco-cigarette dependence alone (Busto, 2009; Fehr, 2008; Martinez, 2005; Volkow, 1996). The major result of this study was that cigarette smoking was associated with lower RAC BP<sub>ND</sub>, independent of drinking status.

The finding that cigarette smoking is associated with low RAC BP<sub>ND</sub> is in line with previous studies that reported lower D<sub>2</sub>/D<sub>3</sub> receptor availability in chronic cigarette smokers relative to non-smokers (Busto, 2009; Fehr, 2008; Stokes, 2011). However, the current results are inconsistent with data from Martinez et al. (2005) which documented reduced striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in detoxified alcoholic subjects, even with matching control subjects for smoking status, as we did in the present study.

**Table 6.** Region of interest (ROI) volumes, all groups. Nontreatment-seeking alcoholic smokers: NTS-S; social-drinking smokers: SD-S; social-drinking non-smokers: SD-NS; left/right: L/R; pre/post-commissural: pre/post; dorsal caudate: DCA; dorsal putamen: DPU; ventral striatum: VST.

\* Main effect of group,  $p < 0.05$ , uncorrected

† Different from SD-S, LSD post-hoc test,  $p < 0.05$

‡ Different from SD-NS, LSD post-hoc test,  $p < 0.05$

Region	Volume (cm <sup>3</sup> )		
	NTS-S (N = 34)	SD-S (N = 21)	SD-NS (N = 26)
	Mean ± SD	Mean ± SD	Mean ± SD
L pre-DCA*	3.10 ± 0.58 <sup>†,‡</sup>	3.50 ± 0.63	3.44 ± 0.46
R pre-DCA*	3.35 ± 0.52 <sup>†,‡</sup>	3.81 ± 0.52	3.73 ± 0.49
L post-DCA*	0.59 ± 0.16 <sup>†,‡</sup>	0.72 ± 0.21	0.72 ± 0.22
R post-DCA*	0.55 ± 0.14 <sup>†,‡</sup>	0.68 ± 0.19	0.68 ± 0.21
L pre-DPU*	2.23 ± 0.31 <sup>†,‡</sup>	2.45 ± 0.23	2.36 ± 0.31
R pre-DPU*	2.52 ± 0.30 <sup>†,‡</sup>	2.75 ± 0.25	2.71 ± 0.31
L post-DPU	2.69 ± 0.43	2.80 ± 0.35	2.69 ± 0.44
R post-DPU	2.53 ± 0.42	2.63 ± 0.31	2.52 ± 0.44
L VST	0.60 ± 0.09	0.65 ± 0.06	0.64 ± 0.11
R VST	0.62 ± 0.08	0.67 ± 0.06	0.67 ± 0.11



In other studies of D<sub>2</sub>/D<sub>3</sub> receptor availability in alcoholics, imbalances in smoking status between alcoholics and controls may have accounted for some reported lower D<sub>2</sub>/D<sub>3</sub> receptor availability in alcoholics (Heinz, 2004; Hietala, 1994; Volkow, 1996; Volkow, 2002). An important difference between the current and previous studies is that we sampled nontreatment-seeking alcoholics from the local community, whereas prior work studied abstinent alcoholics after inpatient detoxification. These are likely two distinct populations of alcohol-dependent subjects. Treatment-seeking alcoholics have more severe alcoholism than nontreatment-seekers (Fein, 2005), have a higher comorbidity of psychiatric disorders (Di Sclafani, 2008), and a greater degree of gray and white matter abnormalities (Gazdzinski, 2008). Thus, it is possible that individuals from a community-based, currently heavy-drinking (and smoking) population may not have apparent deficits in striatal D<sub>2</sub>/D<sub>3</sub> receptor availability when compared to social-drinking controls matched for cigarette smoking status.

Because BP<sub>ND</sub> is a compound index ( $B_{avail}/K_D$ ), lower striatal BP<sub>ND</sub> in smokers relative to non-smokers could be interpreted as either lower numbers of D<sub>2</sub>/D<sub>3</sub> receptors, or higher synaptic/extrasynaptic DA concentration. We speculate that cigarette smoking produces an apparently lower D<sub>2</sub>/D<sub>3</sub> availability via increased striatal DA concentration. This would be consistent with a post-mortem study of chronic cigarette smokers, which reported that DA levels in smokers' striatal tissue were significantly higher compared to non-smokers, whereas D<sub>2</sub> and D<sub>3</sub> receptor levels were not different between groups (Court, 1998). It is possible that smoking-induced increases in striatal DA occur through inhibition of monoamine oxidase (MAO). Dopamine is a substrate for both MAO isoforms (MAO-A and -B), and chronic treatment with either MAO-A or MAO-B inhibitors increases basal striatal DA (Kaseda, 1999; Lakshmana, 1998; Lamensdorf, 1996). Several studies established that platelet MAO activity is lower in current cigarette smokers relative to non-smokers and former smokers (Berlin, 1995; Norman, 1987; Orelund, 1981). Multiple PET studies have demonstrated inhibition of both MAO isoforms (MAO-A and -B) in the brains of smokers (Fowler, 1996a; Fowler, 1996b; Leroy, 2009). Cigarette smoke itself is a potent inhibitor of both MAO isoforms (Yu, 1987), and several of the inhibitory compounds in cigarette smoke extract have been identified, including the  $\beta$ -carbolines harman and norharman (Herraiz, 2005). Collectively, this body of evidence strongly supports the possibility that cigarette smoke increases DA levels, resulting in apparent lower striatal RAC D<sub>2</sub>/D<sub>3</sub> availability in smokers.

Alternatively, it is possible that the nicotine patches worn by a majority of subjects induced DA release. All but seven smokers were administered nicotine patches on the scan day, and studies in the animal literature suggest that nicotine itself may cause measurable DA release (Nisell, 1994; Schiffer, 2001). Three human RAC studies found similar results (Brody, 2006; Brody, 2004; Takahashi, 2008). However, in the two Brody et al. studies, the designs included subjects physically smoking while inside the scanner, and it is possible that the chemosensory properties of smoking a cigarette are central factors contributing to the DA release observed in these studies. In fact, recent data support this possibility: Domino et al. (2012) showed that smoking denicotinized cigarettes causes measurable DA release, indicating that the presence of nicotine may not be a necessary condition for increased DA during the act of smoking. Although Takahashi et al. (2008) reported that chewing nicotinized gum increased striatal DA in cigarette smokers, the design included a placebo condition as the “baseline”. If the placebo condition induced a negative prediction error (nicotine expected from the gum, but not delivered), this could have induced a *decrease* in striatal DA (Yoder et al., 2009) during the placebo condition, producing results that show an apparent “increase” in DA during the nicotinized gum condition (see discussion of design confounds in Yoder et al. (2011c)). There is also strong pharmacological evidence that nicotine itself does not induce DA release measurable by RAC PET. When nicotine was administered intranasally to humans and intravenously to unanesthetized monkeys, there were no significant reductions in RAC binding (Montgomery, 2007; Tsukada, 2002). Finally, our data did not show any evidence of a measurable effect of nicotine patches on BP<sub>ND</sub>. Therefore, we suggest that it is unlikely that the use of nicotine patches was a significant confound in this study.

There are limitations to this retrospective study. As all of our alcoholic subjects were also cigarette smokers, we could not assess the specific contributions of alcohol and cigarette use to D<sub>2</sub>/D<sub>3</sub> availability in this sample. Inclusion of a group of non-smoking alcoholics would be needed to confirm the conclusion that the group differences observed in the current study were due solely to chronic smoking. Also, although every effort was made to screen for use of illicit substances, some subjects tested positive for drugs besides marijuana (2 for cocaine, 2 for opiates) on the day of scanning. Examination of these subjects’ data indicated that the BP<sub>ND</sub> values were well within 2 standard deviations of the group mean, indicating that they were not outliers skewing the results. A history of substance abuse or dependence was an exclusion criteria;

however, we cannot preclude the possibility that prior recreational use of other illicit substances contributed variance to the data. The reduced striatal volumes in the NTS-S group introduces another potential confound, via the risk of partial volume effects on  $BP_{ND}$  (Morris, 2004). NTS-S subjects had smaller striatal volumes (Table 6) than the other two social drinking groups, but there were no differences in striatal RAC  $BP_{ND}$  between the alcoholic smokers and social drinking smokers. If striatal atrophy (and hence, partial volume effect) was the sole source of apparent lower  $BP_{ND}$  between smokers and non-smokers, then we might expect similar levels of atrophy in both smoking groups. However, this was not the case. Therefore, we suggest that striatal atrophy in the alcoholic smokers is an unlikely source of significant variance in our data. Another potential concern is the significantly younger age of the SD-NS subjects compared to the SD-S and NTS-S subjects (Table 3). Age-related decline of striatal  $D_2/D_3$  receptor availability is well-documented, with estimates ranging from 4-8% decrease per decade (for comprehensive review, see Ishibashi, 2009). However, the majority of such studies were conducted with a very wide age range (e.g., from 20-80 years of age) in healthy individuals. In the present work, we did not observe a correlation between  $BP_{ND}$  and age in either social drinking group, perhaps because of the limited age range of our samples. However, we did observe a correlation between age and  $BP_{ND}$  in the smoking alcoholic group (data not shown). Because age was not uniformly related to  $BP_{ND}$  across our samples, we believed it was not appropriate to use age as a covariate in the data analyses. Our observations of age-related decline in  $BP_{ND}$  in NTS-S (especially in a sample with a restricted age range) is consistent with recent evidence, which suggests that this decline may be accelerated in certain populations such as schizophrenia (Kegeles, 2010) and alcoholism (Rominger, 2012a).

In conclusion, the primary finding in the present study is that, regardless of drinking status, cigarette smokers have lower striatal  $D_2/D_3$  receptor availability compared to non-smokers. This is important, as it suggests that some component(s) of cigarette smoke may act on the dopamine system independently of alcohol abuse. This adds to the growing literature demonstrating the adverse effects of cigarette smoking on brain structure and chemistry (for review, see Durazzo, 2007). Additionally, recent findings indicate that continued cigarette use during treatment for substance abuse may be detrimental to clinical outcome (for review, see Kalman, 2010). Although it is tempting to speculate that the effects of cigarette smoking on brain dopamine may interfere with treatment for alcoholism and other addictions, more research is needed to

explore this possibility. In addition to studying the clinical implications of smoking on D<sub>2</sub>/D<sub>3</sub> availability, future studies must address the molecular ramifications of chronic cigarette abuse on the dopamine system.

#### Acknowledgements

Supported by ABMRF/The Foundation for Alcohol Research (KKY), NIAAA 5P60AA007611-25 (pilot P50 to KKY), NIAAA R21AA016901 (KKY), NIAAA R01AA018354 (KKY) and the Indiana Clinical and Translational Sciences Institute (NIH TR000006, Indiana Clinical Research Center).

The authors would like to thank Christine Herring, Lauren Federici, Cari Cox Lehigh, and Elizabeth Patton for assistance with recruitment and data collection; Kevin Perry for acquisition of PET data; Michele Beal and Courtney Robbins for assistance with MR scanning; and Dr. Bruce Mock, Dr. Clive Brown-Proctor, Dr. Qi-Huang Zheng, Barbara Glick-Wilson and Brandon Steele for [<sup>11</sup>C]raclopride synthesis. We also thank Dr. Andrew Saykin, Dr. Gary Hutchins, and Dr. Nicholas Grahame for valuable discussion regarding data analysis.

### **Chapter 3: Cortical dopamine release during a behavioral response inhibition task**

This chapter explores the use of [<sup>18</sup>F]fallypride to determine whether cortical DA release during a response inhibition task can be detected with current PET methods. This pilot, proof-of-principle study was conducted in nine healthy males. On separate days, subjects received one [<sup>18</sup>F]fallypride scan during a “Go” control task and one [<sup>18</sup>F]fallypride scan during a response inhibition task (stop-signal task). Parametric BP<sub>ND</sub> images were generated and analyzed with SPM8.

Results from the voxel-wise analysis indicated significant SST-induced DA release in several cortical regions involved in inhibitory control, including the insula, cingulate cortex, orbitofrontal cortex, precuneus, and supplementary motor area. The regions reported as having significant task-induced DA changes have been consistently observed in fMRI studies to be activated during response inhibition. There was also a significant positive correlation between DA release in the left orbitofrontal cortex, right middle frontal gyrus, right precentral gyrus, and stop-signal reaction time. These data support the feasibility of using [<sup>18</sup>F]fallypride PET to study DA release during a response inhibition task. Future work will use this paradigm to investigate the relationships between DA function and impulsive behavior.

## Introduction

Impulsive behaviors are a hallmark of several forms of psychopathology, including addiction (Jentsch, 1999), as addicts are often unable to restrain the impulse to pursue and consume addictive substances even in the face of detrimental consequences. There are many facets of impulsivity that are relevant for addiction (Evenden, 1999; Perry, 2008), but one of the most frequently-studied aspects is motor inhibition, which is often characterized by the ability to inhibit a prepotent response. Impaired performance on motor inhibition tasks is a common characteristic across addicted and at-risk populations (e.g. Courtney, 2013; Goudriaan, 2006; Li, 2009; Nigg, 2006). Motor response inhibition is often indexed with stop signal reaction time (SSRT) as derived from the stop signal task (SST). SSRT is defined as the time required to withdraw (Stop) a ballistic hand movement (Logan, 1994; Logan, 1984). Specifically, subjects are required to respond quickly to “Go” stimuli, with intermittent “Stop” stimuli signaling the need to withhold that motor response. Subjects with impaired motor inhibition are less able to inhibit their motor response, and thus have longer SSRTs (Lipszyc, 2010).

A number of human functional magnetic resonance imaging (fMRI) studies suggest that successful response inhibition, as indexed by SSRT, is strongly associated with the blood oxygen level dependent (BOLD) signal in a network of fronto-basal ganglia circuitry (Aron, 2006; Chambers, 2006; Congdon, 2010), particularly in the inferior frontal cortex (IFC), anterior insula (AI), anterior cingulate cortex (ACC), presupplementary motor area (pre-SMA), subthalamic nucleus (STN), globus pallidus (GP), and putamen (PUT). Dopamine (DA) is a neurotransmitter that is critical for modulating activity in many of these regions (Frank, 2005). Additionally, a growing body of evidence suggests that cortical dopaminergic neurotransmission plays a substantial role in mediating impulsive behavior, both generally, and specifically with respect to motor response inhibition. Animal studies have demonstrated increases in frontal DA during an impulsive choice task (delay-discounting) (Winstanley, 2006). Selectively altering frontal DA concentrations via lesions increases impulsive choice (Kheramin, 2004), whereas pharmacologically-increased DA reduces impulsive choice (Robinson, 2008), as indicated by shifts in discounting. St. Onge et al. (2011) also recently reported that prefrontal cortex (PFC)-specific blockade of D<sub>2</sub> receptors increased risky choice in rats. In humans, evidence for a link between cortical dopaminergic transmission and

impulsivity is gradually emerging, and several lines of evidence are converging to support such a relationship. Catechol-O-methyltransferase (COMT) is the enzyme responsible for the majority of dopamine catabolism in the frontal cortex (Chen, 2004). A recent human study found that treatment with tolcapone, a COMT inhibitor, was associated with less impulsive choice, presumably via decreases in frontal cortical DA (Kayser, 2012). In line with this, Boettinger et al. (2007) reported that human subjects with a more active form of COMT display relatively higher impulsive behavior. Taken together, these preclinical and human reports have been instrumental in highlighting the importance of dopaminergic signaling in impulsive behavior. However, it is important to note that many of the above studies employed measures of impulsivity distinct from the stop signal paradigm. Although some facets of impulsivity are likely related across different operational definitions, there is evidence to suggest a disconnect between certain impulsive measures, such as the delay discounting and stop signal tasks (Dalen, 2004; De Wit, 2009; Solanto, 2001). Thus, the specific processes by which DA modulates human motor response inhibition processes are still largely unknown.

Human and small animal studies attempting to elucidate the neuropharmacology of SST performance have yielded equivocal results. Several studies reported that atomoxetine (ATM), a selective norepinephrine (NE) reuptake inhibitor, improves SSRT in both humans (Chamberlain, 2009; Chamberlain, 2006) and small animals (Bari, 2009; Bari, 2011; Robinson, 2008). Furthermore, although atomoxetine increases both cortical DA and NE (Bymaster, 2002), its ability to improve SSRT was shown to be unaffected by cortical DA blockade (Bari, 2011). In contrast, D<sub>2</sub>-specific blockade in the dorsal striatum was shown to selectively impair SST performance (i.e. increase SSRT, Eagle, 2011). In a human study, Nandam et al. (Nandam, 2011) reported that the DA transporter blocker methylphenidate (MP), but not ATM, improved SSRT. Similarly, in a separate human study, the D<sub>2</sub>-specific agonist cabergoline improved SSRT, without affecting overall reaction time (Nandam, 2013). These discrepancies across the literature indicate that motor response inhibition is likely under control of several neurotransmitter systems, although interpretation is likely complicated by inter-species differences in anatomy and neurotransmission, as well as the complexity of the cognitive process.

To date, there has been only one comparison of SST performance with an *in vivo* measure of dopamine receptor availability (Ghahremani, 2012), which found significant correlations between baseline dorsal striatal D<sub>2</sub>/D<sub>3</sub> receptor availability and SSRT (as derived from performance outside the scanner). Furthermore, the authors reported that

baseline dorsal caudate D<sub>2</sub>/D<sub>3</sub> receptor availability was correlated with the BOLD signal during SST in the dorsal caudate and several frontal cortical regions (e.g. ACC, IFG, OFC). However, while this investigation provided novel evidence linking SST performance to baseline striatal dopamine D<sub>2</sub>/D<sub>3</sub> receptor availability, the role of cortical dopaminergic neurotransmission during the performance of a behavioral response inhibition task remains unclear. In an attempt to address this issue, we conducted an proof-of-principle study in healthy subjects to determine whether changes in D<sub>2</sub>/D<sub>3</sub> receptor availability (indicative of changes in dopamine) during a SST could be detected using positron emission tomography (PET) and [<sup>18</sup>F]fallypride (FAL). Subjects were scanned under two conditions: one during performance of a SST, and one during performance of a control attention task requiring only “Go” responses. We hypothesized that the SST would induce changes in dopamine in cortical regions similar to those reported in BOLD fMRI studies of response inhibition.

## Methods

All study procedures were approved by the Indiana University Institutional Review Board and performed in accordance with the ethical standards of the Belmont Report. Subjects were recruited by local advertising in the greater Indianapolis area. Written informed consent was obtained after the study was completely described to the subjects. Nine healthy, right-handed, adult men completed study procedures. Subjects underwent a screening interview that included the Edinburgh handedness inventory (Oldfield, 1971), a 30-day Time Line Follow Back (TLFB; Sobell, 1986) calendar for recent drinking, and the Alcohol Use Disorder Identification Test (AUDIT; Saunders, 1993) to screen for risky drinking behavior. Exclusion criteria were: age less than 18 or greater than 45 years of age, contraindications for MRI, current use of medications with central nervous system action, current use of tobacco or recreational drugs, consumption of  $\geq 15$  drinks per week, or  $> 4$  drinks on one occasion, AUDIT scores  $> 8$ , reported history of neurological and/or psychiatric disorders, and a positive urine toxicology screen (Q-10, Proxam) as administered at screening, and on the day of PET imaging. Subjects received two [<sup>18</sup>F]fallypride (FAL) PET scans, conducted on separate days, and with scan order counterbalanced across subjects. The baseline FAL scan was acquired while subjects performed a control attention task. The challenge FAL scan was acquired during performance of a behavioral response inhibition task (stop signal



task, SST). Initiation of the tasks began five minutes prior to FAL injection. Individual task presentation lasted for ~6 minutes. Tasks were presented four times in a row with a ~5 min break between runs. Total task time was ~45 min. During breaks and after completion of the final tasks, a fixation cross was displayed to help maintain subject wakefulness. Tasks were presented to subjects on a computer monitor situated outside the gantry. The monitor screen was fully visible to the subject. Prior to tracer injection, study personnel ensured that the subject was able to easily see, read, and perform the task without significant head movement. Task responses were made via a wireless mouse that was placed on a table adjacent to the scanner bed; tray table position was adjusted for a comfortable height and distance for the subject. Both “Go” and SST tasks were modified versions used by Kareken et al. (2013) and programmed in E-prime 2.0 software (Psychology Software Tools Inc., Sharpsburg, PA).

#### *Stop-signal task*

Four SST task runs were presented to the subjects. Each SST run consisted of a combination of 80 “Go” trials and 40 “Stop” trials. During “Go” trials, subjects were presented with horizontal blue arrows that pointed left or right. Subjects were instructed to press the “left” mouse button for a left arrow, and the “right” mouse button for a right arrow. Subjects were instructed to respond as quickly and accurately as possible. “Stop” trials consisted of a red “up” arrow that appeared immediately after a blue arrow presentation. Subjects were instructed that, when they saw the red arrow, they were to withhold their response to the immediately preceding blue arrow. Across stop trials, an adaptive staircase algorithm adjusted the temporal delay between “Go” and “Stop” stimuli in 50 ms increments, to achieve a target “Stop” inhibition rate of 50%. That is, for each run, the “Stop” signal delay (SSD) time was set initially at 250 ms, and then either increased or decreased by 50 ms after successful or failed “Stop” response, respectively. SSD was programmed to be between 0 ms and 1450 ms. For each subject, average SSD was computed across all four runs, using only the data after the point at which the subject successfully converged to 50% stop inhibition. The mean, median, and standard deviation of reaction time on “Go” trials were calculated only for “Go” trials in which participants responded correctly. In order to calculate stop signal reaction time (SSRT), all Go-RTs were arranged from smallest to largest. The average SSD was then subtracted from that subject’s  $x$ th percentile “Go” RT, where  $x$  corresponds to the stop failure rate (Band, 2003). Thus, if a subject successfully

inhibited their response on 55% of “Stop” trials, the Go-RT corresponding to the 55<sup>th</sup> percentile of the subject’s Go-RT distribution would then be selected, and the average SSD subtracted from this Go-RT to yield SSRT.

#### *Go attention task*

During “Go” trials, subjects were presented with horizontal blue arrows that pointed left or right. Subjects were instructed to press the “left” mouse button for a left arrow, and the “right” mouse button for a right arrow. Subjects were instructed to respond as quickly and accurately as possible.

#### *Image Acquisition*

A magnetized prepared rapid gradient echo (MP-RAGE) magnetic resonance image (MRI) was acquired using a Siemens 3T Trio-Tim for anatomic coregistration and processing of PET data. [<sup>18</sup>F]fallypride (FAL) was synthesized at the Department of Radiology and Imaging Sciences radiochemistry facilities in the Biomedical Research Training Center, according to previously described methods (Gao, 2010). FAL PET scans were acquired on an ECAT HR+ (3D mode; septa retracted). FAL PET scans were initiated with an IV infusion of  $170.63 \pm 33.4$  MBq FAL over the course of 1.5 minutes. Injected mass was  $0.052 \pm 0.03$  nmol/kg. The dynamic PET acquisition was split into two segments for subject comfort (Christian, 2006). The first half of dynamic acquisition was 60 min (6 x 30s, 7 x 60s, 5 x 120s, 8 x 300s). Following this segment, the subject was removed from the scanner for a ~15 min break period to stretch and use the restroom if needed. The second half of dynamic acquisition lasted 50 min (10 x 300s).

#### *Image Processing*

Dynamic PET data were reconstructed with Siemens ECAT software, v7.2.2. Three-dimensional data were rebinned into 2D sinograms with Fourier rebinning. Sinograms were corrected for randoms, scatter, and attenuation, and images were generated with filtered back-projection with a 5mm Hanning filter. MRI and dynamic PET images were converted to Neuroimaging Informatics Technology Initiative (NIFTI) format (<http://nifti.nimh.nih.gov/>) and processed with SPM8. A mean PET image that contained a mixture of blood flow and specific binding was created using the realignment algorithm. This mean PET was coregistered to the subject’s anatomic MRI using the

mutual information algorithm in SPM8. Each frame of PET data was subsequently coregistered to the MRI-registered mean PET image to correct for subject motion. Each subject's MRI was spatially normalized to Montreal Neurological Institute (MNI) space and the transformation matrix obtained from the spatial normalization step was then applied to the motion-corrected PET data from each subject.

### *Voxel-wise Analysis*

Dopamine (DA) D<sub>2</sub>/D<sub>3</sub> receptor availability was indexed with binding potential relative to nondisplaceable binding (BP<sub>ND</sub>), which is operationally defined as  $f_{ND} * B_{avail} / K_D$  (Innis, 2007). The cerebellum (vermis excluded) was used as the reference region (tissue that contains few to no D<sub>2</sub>/D<sub>3</sub> receptors). Individual gray matter cerebellar regions of interest (ROIs) were created for each subject in order to extract cerebellar time activity curves. BP<sub>ND</sub> was estimated at each brain voxel with Logan reference graphical analysis (Logan, 1996) using the cerebellar time activity curve as input function.  $t^*$  was set at 25 data points in “stretched” time. The resulting parametric BP<sub>ND</sub> images were smoothed with an 8mm Gaussian kernel (Costes, 2005; Picard, 2006; Ziolkowski, 2006). In areas of high D<sub>2</sub>/D<sub>3</sub> receptor density, like the striatum, > 2.5 hours of scanning is required to accurately estimate BP<sub>ND</sub> (Christian, 2006; Christian, 2000). As subjects in our study were scanned for approximately 2 hours, we implemented a gray matter mask to exclude the striatum. In addition, parametric BP<sub>ND</sub> image voxels with very low values (< 0.1) were excluded from further analysis to ensure that only reliably estimated BP<sub>ND</sub> values from both scans were considered.

### *Statistical Analysis*

Voxel-wise, one-tailed paired *t*-tests were used to detect significant changes in FAL BP<sub>ND</sub> between scan conditions. Tests were run in both directions to test for both increases and decreases in BP<sub>ND</sub> during the SST relative to the attention task condition. Significant clusters were defined at  $p < 0.005$  (uncorrected) and cluster size  $k > 10$  voxels. Each significant cluster was defined as a region of interest (ROI), and average regional BP<sub>ND</sub> values were extracted from the “Go” baseline and SST parametric images with the MarsBaR toolbox (<http://marsbar.sourceforge.net/>). This allowed us to calculate percent change in BP<sub>ND</sub>:  $(\% \Delta BP_{ND}) = ((BP_{ND, GO} - BP_{ND, SS}) / BP_{ND, GO}) * 100$  for each cluster, and to test for bivariate correlations with SSRT using SPSS 20.0. Data

from significant regression analyses were tested for outliers using Cook's  $D$  (Bollen, 1985). Data are presented as mean  $\pm$  s.d., unless otherwise specified.

## Results

### *Subject Characteristics*

Subjects were  $24.6 \pm 4.1$  years old (range 19 – 32), and had  $15.7 \pm 1.3$  years of education. All subjects were light social-drinkers: average alcohol consumption was  $1.91 \pm 2.5$  drinks per week; AUDIT scores were  $3.0 \pm 1.7$ .

### *Task performance*

Behavioral results from the “Go” control attention task and SST are shown in Table 7. Data for the “Go” task from one subject was unavailable because of computer failure. Behavioral SST data from two subjects were excluded because they failed to converge to 50% stop inhibition throughout the course of the task.

### *Changes in FAL $BP_{ND}$ during the Stop Signal Task*

Voxel-wise paired  $t$ -tests revealed several cortical regions where  $BP_{ND}$  during the SST ( $BP_{ND, SS}$ ) was significantly lower than  $BP_{ND}$  during the “Go” attention task ( $BP_{ND, GO}$ ) (Figure 4, Table 8), indicative of dopamine (DA) release in these regions during the SST.  $BP_{ND, GO}$  was significantly lower than  $BP_{ND, SS}$  in the anterior cingulate gyrus (Figure 5, Table 8), indicative of decreased DA in this region during the SST.

### *Association between $\Delta BP_{ND}$ and Stop Signal Task performance*

Of the 21 extracted clusters in which there was a significant change in  $BP_{ND}$  (Figures 4 – 5, Table 8),  $\Delta BP_{ND}$  significantly negatively correlated with SSRT ( $n = 7$ ) in the left orbitofrontal cortex (OFC;  $r = -0.842$ ,  $p = 0.017$ ), right middle frontal gyrus (MFG;  $r = -0.833$ ,  $p = 0.020$ ), and right precentral gyrus ( $r = -0.877$ ,  $p = 0.009$ ). None of the data points in any of the three regressions met the criteria as undue influence points, which was defined by threshold  $D < 0.57$ .  $D$ -value ranges were: L-OFC, 0.000 – 0.268; R-MFG, 0.017 – 0.432; R-precentral gyrus, 0.015 – 0.403.

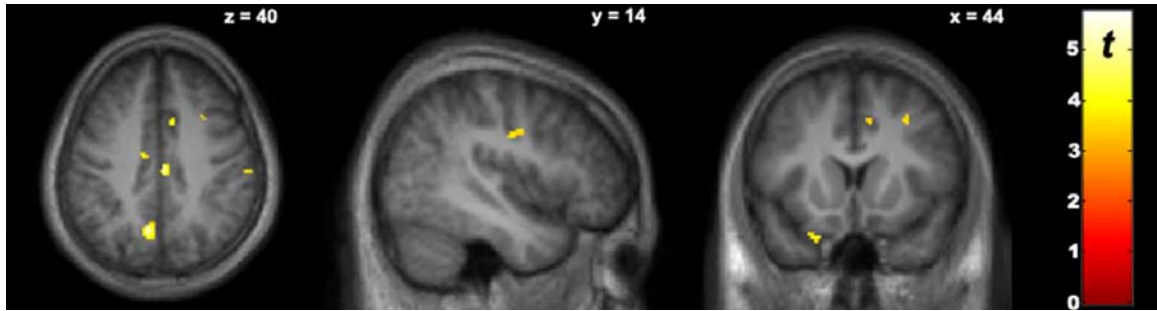
**Table 7.** Performance on the “Go” attention task and Stop Signal task. Data are mean  $\pm$  s.d. RT, reaction time; SSRT, stop signal reaction time,

"Go" task performance (n = 8)	
Correct trials (%)	98.5 $\pm$ 1.4
Median Go-RT (ms)	378 $\pm$ 45
Stop Signal task performance (n = 7)	
Correct "Go" trials (%)	96.5 $\pm$ 7.6
Correct "Stop" trials (%)	56.3 $\pm$ 9.5
Median Go-RT (ms)	577 $\pm$ 140
SSRT (ms)	232 $\pm$ 23

**Table 8.** Voxel-wise results of changes in dopamine (DA) during the SST. Regions of increased DA were taken from the  $BP_{ND, BL} > BP_{ND, SS}$  contrast. Regions of decreased DA were taken from the  $BP_{ND, SS} > BP_{ND, BL}$  contrast. MNI, Montreal Neurological Institute;  $k$ , cluster size; STG, superior temporal gyrus; IPL, inferior parietal lobe; SMG, supramarginal gyrus; SMA, supplementary motor area; ITG, inferior temporal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus; IFG, inferior frontal gyrus; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex. Statistical threshold was  $p < 0.005$ , uncorrected,  $k > 10$  voxels.

Region/cluster	MNI Coordinates			Cluster Size ( $k$ )	Peak voxel (Z-score)	% $\Delta BP_{ND}$ Mean $\pm$ SD
	$x$	$y$	$z$			
Regions of increased DA						
Precuneus	-8	-62	36	141	3.52	10.2 $\pm$ 5.7
	14	-58	62	33	3.30	15.2 $\pm$ 12
Cingulate cortex	4	-20	40	14	3.34	5.9 $\pm$ 3.6
	10	12	42	22	3.00	9.3 $\pm$ 6.5
	-10	-12	40	11	2.81	6.5 $\pm$ 4.0
IPL/SMG	64	-36	34	28	3.33	12.1 $\pm$ 3.8
SFG/SMA	-10	4	58	23	3.28	8.2 $\pm$ 5.2
Fusiform gyrus	-28	-50	-16	25	3.21	5.9 $\pm$ 4.0
	-40	-38	-26	45	3.12	15.5 $\pm$ 11
STG	60	-50	14	21	3.18	6.5 $\pm$ 5.0
	62	-36	12	51	3.06	17.1 $\pm$ 8.3
Angular gyrus	50	-62	30	26	3.15	7.1 $\pm$ 5.0
Paracentral lobule	6	-32	68	22	3.06	11.6 $\pm$ 9.0
Postcentral gyrus	62	-14	16	85	3.01	8.2 $\pm$ 5.4
MFG	30	10	44	37	2.99	19.0 $\pm$ 15
	20	-16	64	22	2.77	24.8 $\pm$ 19
	36	24	48	26	3.34	11.2 $\pm$ 8.2

OFC/IFG	-22	14	-28	13	3.00	6.80 ± 4.1
Precentral gyrus	46	-10	32	22	2.81	16.2 ± 13
Uncus	-32	-14	-38	10	3.01	14.5 ± 10
Regions of decreased DA						
ACC	-2	30	-4	16	3.13	-33.9 ± 32



**Figure 4.** Whole-brain voxel-wise paired  $t$ -test comparing  $BP_{ND}$  between baseline “Go” and SST scan conditions ( $n = 9$ ). The “hot” colorscale indicates voxels where  $BP_{ND, BL}$  was significantly higher than  $BP_{ND, SS}$  (increased DA during SST). Display threshold  $p < 0.005$ , uncorrected,  $k > 10$ . Significant clusters are listed in Table 8.





**Figure 5.** Whole-brain voxel-wise paired  $t$ -test comparing  $BP_{ND}$  between baseline “Go” and SST scan conditions ( $n = 9$ ). The “cool” colorscale indicates voxels where  $BP_{ND, SS}$  was significantly higher than  $BP_{ND, BL}$  (indicating decreased DA during SST). Display threshold  $p < 0.005$ , uncorrected,  $k > 10$ . Significant clusters are listed in Table 8.

## Discussion

The principle finding of the current study is that changes in cortical D<sub>2</sub>/D<sub>3</sub> receptor availability were detectable during a stop signal task (SST) relative to a control attention task. To our knowledge, this is the first demonstration of *in vivo* changes in cortical DA during a motor response inhibition task. The anatomic locations of significant clusters of  $\Delta BP_{ND}$  correspond well to neural correlates of inhibiting motor responses that have been characterized in humans with fMRI (Aron, 2006; Chambers, 2009; Congdon, 2010). These, and other reports, have emphasized the importance of the IFC, SMA, pre-SMA, ACC, STN, and striatum in successful response inhibition (Zandbelt, 2010). While fMRI provides excellent spatial localization and has good temporal sampling ability, other *in vivo* techniques such as PET are needed to elucidate the specific neurochemical substrates of the SST. Using [<sup>18</sup>F]fallypride (FAL) PET, we demonstrated SST-induced changes in dopaminergic signaling in several cortical regions that are implicated in behavioral response inhibition. In particular, we observed significant increases in DA in motor-related brain regions such as the SMA and precentral gyrus (Figure 4, Table 8), which are thought to be crucial regions in the stopping process (Floden, 2006; Li, 2006). Other cortical regions that exhibited significant SST-induced changes in DA have previously been shown to activate during SST performance, including frontal (middle and superior frontal gyri), parietal (precuneus, paracentral lobule, postcentral gyrus, supramarginal gyrus, angular gyrus), temporal (fusiform gyrus, superior temporal gyrus) and cingulate cortex areas (Cai, 2009; Congdon, 2010; Courtney, 2013; Ghahremani, 2012; Kareken, 2013).

The precuneus is one of the core regions of the “default mode network” (DMN; Bressler, 2010), which engages in the absence of a directed task and is believed to mediate “switching” cognitive processes on and off (Li, 2007; Zhang, 2010). Dopaminergic transmission affects precuneus activity during cognitive task performance. For example, Argyelan et al. (2008) showed that cognitively-induced change in precuneus activity was affected by a DA agonist. Tomasi et al. (2009) found that deactivation of the precuneus during a visuospatial attention task was negatively associated with striatal dopamine transporter availability. The SST-related increases in dopamine in the precuneus that were observed in this study may indicate a role for dopamine in deactivating the DMN in order to engage processes relevant for motor response task performance.

In the present analysis, we also report that task-induced changes in D<sub>2</sub>/D<sub>3</sub> receptor availability were negatively correlated with SSRT in three cortical subregions, the left orbitofrontal cortex, right middle frontal gyrus, and right precentral gyrus (Table 8). These regions have been identified as belonging to a common network that exhibits SST-induced activation, in which activation was also significantly associated with SSRT (Congdon, 2010). Another fMRI study confirmed that SST-induced BOLD responses in these regions were associated with SSRT (Ghahremani, 2012). These reports lend support to our interpretation of the present data, which is that cortical dopamine in these regions may contribute to performance of a motor response inhibition task. However, the literature on the cortical neurochemistry and neuroanatomy underlying motor inhibition performance is admittedly more complicated.

Human imaging studies have not definitively discerned the precise locations and neurotransmitter systems relevant for response inhibition. There is much debate over which specific brain regions are important for stopping a motor response. While numerous studies cite the IFC as an essential locus for successful inhibition (Aron, 2003; Swick, 2008), there is also evidence supporting the OFC (Horn, 2003), and precentral gyrus (Li, 2006) as neural hubs for response inhibition modulation. A recent fMRI study in adolescents reported differential activation of brain networks during a SST that was dependent on substance abuse and ADHD phenotypes (Whelan, 2012). This suggests that interindividual variability may affect which specific brain regions are recruited for task performance.

Global manipulations of DA function provide indirect evidence about dopaminergic regulation of brain activity during impulse control. Administration of a DA agonist increases regional cerebral blood flow (rCBF) in the OFC and precentral gyrus, and decreases rCBF in the MFG (Bradberry, 2012), indicating that activity in these regions is under control of DA transmission. During motor inhibition tasks, DA perturbation has similar effects on brain activity. DA antagonism results in decreased activation of the precentral gyrus during a motor inhibition task (Luijten, 2013). Conversely, increasing brain DA levels leads to increased activation of MFG and precentral gyrus during a SST (Li, 2010). Furthermore, the change in MFG activation is positively correlated with improvement of SSRT, suggesting that DA may be an important modulator of MFG activity during SST performance. Overall, the data from the literature, combined with that from the current study, suggest that the association of OFC, MFG, and precentral gyrus activation with performance on a response inhibition

task may be modulated, in part, by DA. However, replication of these results in a larger cohort is necessary to further understand these associations.

The present study has several limitations. The sample size is relatively small, and thus presents a risk of both Type I and Type II errors. We readily acknowledge that this is a preliminary analysis, and replication in a larger sample is needed to support our interpretations. However, task-induced DA changes in this study overlap well with regions that have been demonstrated to elicit BOLD responses during a stop signal task (Aron, 2006; Chambers, 2009; Congdon, 2010). This suggests that our findings are physiologically relevant and not merely the result of a Type I error. Another potential limitation of the current study is that we did not examine the striatum. Ghahremani et al. (2012) recently reported that baseline striatal D<sub>2</sub>/D<sub>3</sub> receptor availability was negatively correlated with SSRT. Additionally, several fMRI studies have shown striatal activation during successful response inhibition (Congdon, 2010; Vink, 2005; Zandbelt, 2010). However, because we were primarily interested in cortical DA, our study design used a 2-hour scan: long enough to accurately estimate cortical, but not striatal BP<sub>ND</sub>.

In conclusion, we detected significant changes in cortical D<sub>2</sub>/D<sub>3</sub> receptor availability during a stop signal task compared to a control attentional task. Percent change in receptor availability was correlated with task performance in three cortical regions that have been shown to be important for successful response inhibition. The present results demonstrate the feasibility of using [<sup>18</sup>F]fallypride PET to detect changes in DA during a stop signal challenge, and the potential to use the SST as a probe for studying cortical dopaminergic contributions to disorders marked by impulsive behavior.

#### Acknowledgements

Supported in part by the Indiana University-Purdue University at Indianapolis Research Support Funds Grant to KKY.

The authors would like to thank Christine Herring and James Walters for assistance with recruitment and data collection; Kevin Perry for acquisition of PET data; Michele Beal and Courtney Robbins for assistance with MR scanning; and Dr. Bruce Mock, Dr. Clive Brown-Proctor, Dr. Qi-Huang Zheng, Barbara Glick-Wilson and Brandon Steele for [<sup>18</sup>F]fallypride synthesis. We also thank Dr. Bill Eiler for help coding the stop-signal paradigm in E-prime 2.0.

## Summary

The results of this report highlight the utility of PET imaging as a tool for probing the DA system in addicted populations and addiction phenotypes. This technique offers a unique ability to investigate *in vivo* DA function in awake humans, as well as allowing for comparison of PET metrics (binding potential;  $BP_{ND}$ ) with subjective self-report and behavioral measures. The first chapter describes the use of [ $^{11}C$ ]raclopride (RAC) PET to examine the baseline striatal DA system in chronic cannabis users. The results support previous findings in similar cohorts, showing that chronic cannabis use is not associated with lower striatal  $D_2/D_3$  receptor availability, unlike reports for several other drugs of abuse. Urine levels of THC and THC metabolites were correlated with self-report of recent cannabis consumption, confirming that urine metabolite levels can provide an accurate estimate of recent use. Unexpectedly, both urine THC and THC metabolite levels, as well as self-reported recent use, were significantly negatively associated with RAC  $BP_{ND}$  in the bilateral dorsal putamen. This is the first evidence to link magnitude of recent substance abuse of any drug with  $D_2$  receptor availability. The association between striatal  $D_2/D_3$  receptor availability and recent cannabis consumption was preliminarily interpreted as a result of smoking-induced monoamine oxidase (MAO) inhibition. Overall, the results reported in Chapter 1 support previous literature in cannabis users suggesting that the relationship between cannabis abuse and  $D_2$  receptor availability is different from that of other drugs of abuse. The prevalence of cannabis abuse and dependence is rising, and these findings may have treatment-relative applications. That chronic cannabis abuse appeared to affect the DA system differently than other abused drugs indicates that perhaps different therapeutic strategies should be employed to treat individuals suffering from cannabis dependence. Further studies are necessary to explore the relationship between baseline DA state and recent cannabis consumption.

The second chapter of this report describes a retrospective analysis of baseline striatal  $D_2/D_3$  receptor availability in three distinct groups: nontreatment-seeking alcoholic smokers (NTS-S), social-drinking smokers (SD-S), and social-drinking non-smokers (SD-NS). All subjects received a RAC PET scan at rest. All but seven smoking subjects received a transdermal nicotine patch during the scan day to minimize craving. The observed results were contrary to our initial hypothesis, such that smokers had lower  $D_2/D_3$  receptor availability compared to non-smokers, regardless of drinking status.

SD-NS subjects were significantly younger than smoking subjects. However, when the association of age with RAC BP<sub>ND</sub> was compared for each group separately, there was a significant correlation between age and striatal BP<sub>ND</sub> only in the NTS-S group. Because there was a significant group\*age interaction, the use of age as a covariate across all groups was deemed invalid (see Chapter 2 Discussion, p. 47). The significant negative association of age with striatal BP<sub>ND</sub> only in NTS-S subjects is potentially of interest. This suggests that age-related reduction of D<sub>2</sub> receptors associated with normal aging may be accelerated in a population of alcohol and tobacco-abusing individuals. Further studies are needed to determine whether comorbid alcohol and tobacco abuse might exacerbate normal age-related decline of striatal D<sub>2</sub>/D<sub>3</sub> receptor availability. In order to determine what effects, if any, nicotine patch administration had on striatal BP<sub>ND</sub>, one-way ANOVA was utilized to detect differences in striatal BP<sub>ND</sub> between three subsets of smokers, matched for age and drinking status: smokers who did not receive a nicotine patch during scan day, smokers who received a patch dose of 7mg or 14mg nicotine, and those who received a patch dose of 21mg nicotine. There were no main effects of patch dose on striatal BP<sub>ND</sub>, indicating a negligible effect of nicotine patch on our reported striatal BP<sub>ND</sub> values. Additionally, in line with previous reports in alcoholics, NTS-S subjects exhibited significantly lower striatal volumes than social-drinking groups. However, because RAC BP<sub>ND</sub> was not different between NTS-S and SD-S groups, we concluded that striatal atrophy in NTS-S subjects did not significantly contribute to group differences in BP<sub>ND</sub>. Overall, the results from this study suggest that some non-nicotine component(s) of cigarette smoke may act on the DA system independently of alcohol abuse. We interpret the association of reduced BP<sub>ND</sub> in smokers compared to non-smokers as evidence of smoking-induced inhibition of MAO, which results in increased tonic DA levels. Further studies are needed to address the molecular ramifications of chronic cigarette abuse on the DA system.

The final chapter of this document describes a pilot study to determine if changes in cortical DA induced by response inhibition task can be detected by [<sup>18</sup>F]fallypride (FAL) PET. Nine healthy, social-drinking males received two FAL scans on separate days. One scan was conducted during performance of a “Go” control task, and the other was conducted during performance of a stop signal task (SST). This task assesses the ability to withhold a prepotent motor response, which is indexed by stop signal reaction time (SSRT). SSRT is defined as the time required to withdraw (Stop) a ballistic hand movement. Parametric BP<sub>ND</sub> images were generated using the Logan graphical

technique.  $BP_{ND}$  and images for the separate “Go” and SST conditions were compared to determine regions that exhibited changes in DA during the SST task compared to the control “Go” task. The results indicate that SST performance induced changes in DA in several cortical regions. The regions in which SST-induced DA changes were observed correspond well to neural correlates of inhibiting an ongoing motor response that have been identified by human fMRI studies. Additionally, we explored an association between  $\Delta BP_{ND}$  in cortical regions that exhibited significant SST-induced changes in DA, and SST performance (SSRT).  $\Delta BP_{ND}$  in the left orbitofrontal gyrus, right middle frontal gyrus, and right precentral gyrus was negatively correlated with SSRT. These results indicate that DA may be important for modulating activity in these regions during performance of a response inhibition task. Overall, the results presented in Chapter 3 demonstrate the feasibility of utilizing FAL PET to detect cortical DA changes during a SST challenge. This provides an important foundation for using SST to investigate DAergic contributions to disorders characterized by impulsive behavior, such as addiction.

In summary, this report details the use of DAergic PET to characterize the DA system in addiction. PET imaging, along with DAergic ligands such as RAC and FAL, is a useful tool for probing both the DA system at baseline or “rest” (Chapters 1 and 2), as well as the DAergic response to a challenge condition (Chapter 3). In Chapters 1 and 2, we used PET and the  $D_2/D_3$  antagonist RAC to show that recent cannabis consumption and tobacco-smoking are both associated with reduced  $D_2/D_3$  receptor availability. We tentatively interpret these effects as evidence of smoking-induced inhibition of MAO in the brain, leading to subsequent elevations of DA. These effects appear to be independent of the pharmacological actions of THC and nicotine alone. Given the extreme prevalence of tobacco smoking among populations of currently using and rehabilitated addicts, these results have important implications for clinical treatment of any kind of addiction associated with comorbid tobacco use. Perhaps concurrent treatment for tobacco abuse might improve treatment outcome for other disorders. Furthermore, in agreement with our results, nicotine replacement therapy has limited efficacy for tobacco smoking cessation, which could be due to its limited effects on striatal DA concentrations. Future treatment options involving manipulations of striatal DA tone could prove effective for treatment of tobacco dependence as well as comorbid addictions. Results from Chapter 3 emphasize the use of an additional PET measure to characterize addictive phenotypes: changes in  $BP_{ND}$  in response to a cognitive

challenge. The current protocol was carried out in non-addicted, social-drinkers, but it serves as a stepping-stone for similar studies to be undertaken in addicted populations. Potentially, this has broad treatment applications, as it could be used to identify neural loci where DA signaling during response inhibition is dysfunctional in addicts. Though the utility of PET as a diagnostic tool for addictions is presently impractical, the current results underscore the relevance of PET imaging as a tool for characterizing the DA system in addiction.



## Future Directions

Results from the current report have yielded a great deal of novel information about the DA system in human addiction. Additionally, they establish a basis on which new research questions can be interrogated. One such research question I would like to further explore is how DA levels are modulated by tobacco smoke. Based on results in the current document, we suggest that smoking-induced inhibition of MAO is responsible for the lower RAC BP<sub>ND</sub> observed in our smoking cohorts. However, without gathering additional data, this interpretation remains speculative. To further investigate this issue, I propose additional analyses in a separate sample of tobacco smokers. Briefly, a group of tobacco smokers would be given one RAC scan at rest, as previously described. Urine and blood samples would be collected during the scan day. Urine nicotine and cotinine concentrations would be quantified in order to estimate recent tobacco consumption, which would be assessed for agreement with self-report. Platelet MAO activity would be assayed to determine the extent of inhibition (Murphy, 1976). If smoking-induced inhibition of MAO is responsible for elevated striatal DA concentrations, then I would expect MAO activity to be positively correlated with striatal BP<sub>ND</sub>. This finding would lend credence to the hypothesis that low striatal RAC BP<sub>ND</sub> observed in our smokers is directly related to smoking-induced MAO inhibition. However, the absence of such a relationship would not preclude validation of the hypothesis. There is evidence to suggest that brain MAO occupancy may not be correlated with platelet MAO activity in certain circumstances [(Fowler, 2003; Young, 1986), although see (Bench, 1991)], and this would confound results from the proposed study. One way to conclusively determine brain MAO activity would be to conduct additional PET scans with MAO radiotracers [<sup>11</sup>C]clorgyline and [<sup>11</sup>C]deprenyl-D2 to estimate activities of MAO-A and MAO-B, respectively. However, the excessive cost of this analysis would likely be prohibitive. Alternatively, a genetic component could be explored. Recently, MAO-A polymorphisms that produce “high-” and “low-function” alleles have been associated with antisocial behavior, impulsivity, and other neuropsychiatric disorders (Craig, 2007). It would be interesting to see if a genetic trait associated with behavioral disorders is correlated with DA receptor availability. Although there is likely a disconnect between MAO-A genotype and brain MAO-A activity (Fowler, 2007), recent evidence suggests that methylation patterns of the *MAOA* gene promoter can predict brain MAO-A activity (Shumay, 2012). Information about the relationship

between MAO activity and striatal D<sub>2</sub>/D<sub>3</sub> receptor availability would benefit studies of tobacco, alcohol, and illicit drug use disorders, as well as other neuropsychiatric disorders.

An additional research area I would like to further explore is the apparently accelerated rate of D<sub>2</sub> receptor decline in co-abusers of alcohol and tobacco (Chapter 2 discussion). If the heavy co-abuse of alcohol and tobacco is related to an exacerbation of the age-related decline of D<sub>2</sub> availability associated with normal aging, this could have broad clinical implications, as it would suggest an interaction effect of alcohol and tobacco on the DA system over time. With careful recruitment, this question could be addressed concomitantly with the previously proposed analysis of RAC PET in the same cohort of smokers. An additional group of non-smoking alcoholics would be necessary in order to parse the effects of chronic alcohol drinking alone, without comorbid tobacco abuse. It would also be important to enroll subjects with a sufficient age range to better be able to detect an age effect on RAC BP<sub>ND</sub>. Regression analyses would be performed between age and striatal BP<sub>ND</sub> for each group, using the ten anatomically-defined striatal regions previously discussed (Chapters 1-2). Because the normal age-related decline of D<sub>2</sub> receptors is well documented, I would expect significantly negative regressions between age and D<sub>2</sub>/D<sub>3</sub> receptor availability in all groups. If indeed there is an interaction effect between chronic alcohol and tobacco abuse on the DA system, I would expect the slope from the regression of age with striatal RAC BP<sub>ND</sub> to be significantly steeper than regression slopes from the other groups. It is possible that this analysis would not detect a significant difference between regression slopes. In that event, I would conduct a principal components analysis in an attempt to identify striatal networks whose BP<sub>ND</sub> values were collinearly associated with age. This would identify striatal regions whose BP<sub>ND</sub> values were similarly affected by age, and may grant us additional power to detect main effects of group. Because the effect of age on striatal D<sub>2</sub> receptor availability is heterogeneous across striatal regions (Ishibashi, 2009; Kim, 2011), this type of analysis might yield novel relationships between striatal subregions that are affected similarly by alcohol and tobacco co-abuse. Additionally, it would be interesting to also compare rates of age-related decline of extrastriatal D<sub>2</sub> receptors with [<sup>18</sup>F]fallypride PET. Similar to well-documented age-related reductions in striatal D<sub>2</sub> availability, cortical D<sub>2</sub> receptor availability is also subject to reductions over time (Inoue, 2001; Kaasinen, 2000). Interestingly, there is preliminary evidence suggesting that age-related decline of extrastriatal D<sub>2</sub> availability is accelerated in alcoholic subjects

(Rominger, 2012a), although the individual effects of alcohol and tobacco use could not be discerned by the study design. The potentially separable effects of alcohol and tobacco on age-related  $D_2$  reductions could be investigated by inclusion of a group of non-smoking alcoholic subjects, or a group of non-drinking smokers without a past history of alcohol or substance abuse. Knowledge of the striatal and extrastriatal regions that are most sensitive to alcohol and tobacco-enhanced  $D_2$  decline would be helpful to identify those regions, and potentially, individuals, that are most at risk for damage due to chronic use of alcohol and tobacco.

One final research question I would like to ask is: what are the neural correlates of response inhibition in alcoholics, and how do they differ from social drinkers? To answer this, I would conduct a FAL PET study similar to the one described in Chapter 3. Briefly, the study would include a group of nontreatment-seeking alcoholics and a group of social-drinking controls, matched for age, sex, and smoking status. Subjects would receive two FAL scans on separate days, one during a “Go” control task and one during a SST. Scans would be acquired for 180 minutes in order to accurately estimate striatal  $BP_{ND}$  with FAL. The data would then be analyzed to detect group differences in SST-induced DA release. If results from the control subjects in the proposed study replicated the results presented in Chapter 3, this would greatly strengthen the validity of the results presented here. Evidence of group differences in SST-induced DA release would be helpful in identifying the neural locus of impaired impulsive control in alcoholics. Going further, it would be of interest to compare changes in FAL  $BP_{ND}$  between scan conditions to measures derived from an additional imaging modality. One imaging technique that could be employed is diffusion tensor imaging (DTI). DTI has identified TBI-associated reductions in white matter tract integrity (indexed by fractional anisotropy) that correlated with SST performance (Bonnelle, 2012). Resting state fMRI (RS-fMRI) is another potentially applicable modality. RS-fMRI signals in the IFC and default mode network hubs are predictive of SST performance (Tian, 2012). In conjunction with FAL PET, DTI and RS-fMRI could provide novel information about how differences in structural connections can regulate DA signaling during a motor inhibition task.

Results from the current report have provided important information about DA function in addiction and addictive phenotypes. However, further studies are necessary to both validate the present data, as well as address questions that have arisen from our interpretation of the data.

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selective assessment of phasic dopamine-dependent behavior. *Proceedings of the National Academy of Sciences*, 106(18), 7281-7288

## Curriculum Vitae

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Wabash College, Crawfordsville, IN
- February 2014 Ph.D.  
Medical Neurosciences Graduate Program  
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#### HONORS, AWARDS, FELLOWSHIPS:

Campbell-Klatte Lecture Travel Award Winner, 2013  
Larry Kays Fellowship, 2012  
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IUPUI GSO Educational Enhancement Grant, 2011  
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Research Society of Alcoholism Student Merit Travel Award, 2009  
Indiana University Pre-doctoral Fellowship, 2007  
Howell Undergraduate Chemistry Award, 2005  
Wabash College Presidential Scholarship, 2003

#### AFFILIATIONS:

Phi Beta Kappa, 2007  
Phi Lambda Upsilon Chemistry Honor Society, 2007

#### PROFESSIONAL SOCIETIES:

Research Society on Alcoholism, 2007-present  
Society for Neuroscience, 2011-present  
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#### PEER-REVIEWED DATA-BASED PUBLICATIONS:

1. **DS Albrecht**, DA Kareken, KK Yoder (2013). Effects of smoking on D<sub>2</sub>/D<sub>3</sub> striatal receptor availability in alcoholics and social drinkers. *Brain Imagin Behav* Sept 7(3); 326-34.
2. BG Oberlin, M Dzemidzic, SM Tran, CM Soeurt, **DS Albrecht**, KK Yoder DA Kareken (2013). Beer flavor provokes striatal dopamine release in male drinkers: mediation by family history of alcoholism. *Neuropsychopharmacology* August 38(9); 1617-24.
3. **DS Albrecht**, PD Skosnik, JM Vollmer, MS Brumbaugh, KM Perry, BH Mock, QH Zheng, LA Federici, EA Patton, CM Herring, KK Yoder (2013). Striatal D<sub>2</sub>/D<sub>3</sub>

receptor availability is inversely correlated with cannabis consumption in chronic cannabis users. *Drug Alcohol Depend* Feb 128(1-2); 52-7.

4. KK Yoder, **DS Albrecht**, DA Kareken, LM Federici, KM Perry, EA Patton, QH Zheng, BH Mock, S O'Connor, CM Herring (2012). Reliability of striatal [<sup>11</sup>C]raclopride binding in smokers wearing transdermal nicotine patches. *Eur J Nucl Med Mol Imaging* Feb;39(2):220-5.
5. A Anand, G Barkay, M Dziedzic, **D Albrecht**, H Karne, Q-H Zheng, GD Hutchins, MD Normandin, KK Yoder (2011). Striatal Dopamine Transporter Availability in Unmedicated Bipolar Disorder. *Bipolar Disorders* Jun;13(4):406-13.
6. KK Yoder, **DS Albrecht**, DA Kareken, LM Federici, KM Perry, EA Patton, QH Zheng, BH Mock, S O'connor, CM Herring (2011). Test-retest variability of [<sup>11</sup>C]raclopride binding potential in nontreatment-seeking alcoholics. *Synapse* Jul;65(7):553-61.

#### **MANUSCRIPTS IN PREPARATION OR UNDER REVIEW:**

1. **DS Albrecht**, P MacKie, BT Christian, C Brown-Proctor, LM Federici, DA Kareken, CM Herring, JW Walters, KK Yoder (2013). [<sup>18</sup>F]Fallypride receptor availability in fibromyalgia. [manuscript in preparation]
2. KK Yoder, **DS Albrecht**, CM Herring, LM Federici, EA Patton, SJ O'Connor, DA Kareken. (2013) Changes in Striatal Dopamine in Response to IV Alcohol in Nontreatment-Seeking Alcoholics but not Social Drinkers [manuscript in preparation]
3. **DS Albrecht**, DA Kareken, BT Christian, KK Yoder (2013). Cortical dopamine release during a behavioral response inhibition task. *Under Review*.

#### **INVITED PRESENTATIONS:**

1. **DS Albrecht** (2011) "Striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in chronic marijuana users: association with cannabis consumption and craving." *Addiction Psychiatry Symposium*, Indianapolis, IN. January 25, 2011.
2. **DS Albrecht** (2012) "Factors affecting the dopamine system in addiction." 3<sup>rd</sup> Annual Ann Daugherty Symposium: For Basic Science and Addiction Recovery. Tara Treatment Center, Franklin, IN. June 8, 2012.
3. **DS Albrecht** (2013) "Cortical dopamine release during a response inhibition task in social drinkers." *Center for Neuroimaging Roundtable Discussion*. Indianapolis, IN. May 20, 2013.

#### **ABSTRACTS:**

1. **DS Albrecht**, P MacKie, BT Christian, KK Yoder. *Differences in dopamine function in fibromyalgia*. Submitted for the 10<sup>th</sup> International Symposium on Functional Neuroreceptor Mapping of the Living Brain. Amsterdam, Netherlands, May 2014.



2. **DS Albrecht**, BG Oberlin, M Dzemidzic, CM Herring, JW Walters, KL Hile, SJ O'Connor, DA Kareken, KK Yoder. *Impulsive choice and alcohol-induced dopamine release in alcoholics and social drinkers*. Submitted for the 37<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference. Bellevue, Washington, June 2014.
3. KK Yoder, **DS Albrecht**, M Dzemidzic, CM Herring, JW Walters, KL Hile, SJ O'Connor, DA Kareken. *Differential dopamine responses to expected IV alcohol in nontreatment-seeking alcoholics and social drinkers*. Submitted for the 37<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference. Bellevue, Washington, June 2014.
4. BG Oberlin, M Dzemidzic, SM Tran, CM Soeurt, **DS Albrecht**, SJ O'Connor, KK Yoder, DA Kareken. *Lateralized nucleus accumbens dopamine release to beer self-administration in male heavy drinkers*. Submitted for the 37<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference. Bellevue, Washington, June 2014.
5. **DS Albrecht**, PD Skosnik, JM Vollmer, MS Brumbaugh, KM Perry, BH Mock, QH Zheng, LA Federici, EA Patton, CM Herring, KK Yoder. *Urine THC metabolite levels correlate with striatal D<sub>2</sub>/D<sub>3</sub> receptor availability*. Indiana Neuroimaging Symposium, Bloomington, IN, October 2013.
6. **DS Albrecht**, DA Kareken, KK Yoder. *Effects of smoking on striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in alcoholics and social drinkers*.  
-Indianapolis Society for Neuroscience, Indianapolis, IN, October 2013.  
-International Conference on Applications of Neuroimaging to Alcoholism, New Haven, CT, February 2013.
7. **DS Albrecht**, P MacKie, BT Christian, C Brown-Proctor, LM Federici, DA Kareken, CM Herring, JW Walters, KK Yoder. *[<sup>18</sup>F]fallypride receptor availability in fibromyalgia*. IUPUI Imaging Research Symposium, Indianapolis, IN, October 2013.
8. KK Yoder, **DS Albrecht**, CM Herring, DA Kareken. *Dopaminergic coding for alcohol-related negative prediction errors in alcoholics but not social drinkers*. IUPUI Imaging Research Symposium, Indianapolis, IN, October 2013.
9. **DS Albrecht**, DA Kareken, BT Christian, C Brown-Proctor, WJ Eiler, M Dzemidzic, CM Herring, JW Walters, KK Yoder. *Cortical dopamine release during a behavioral response inhibition task in social drinkers*.  
-International Conference on Applications of Neuroimaging to Alcoholism, New Haven, CT, February 2013.  
-35<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Francisco, CA June 2012.
10. BG Oberlin, M Dzemidzic, SM Tran, SM Soeurt, **DS Albrecht**, KK Yoder, DA Kareken. *Beer flavor induces orbitofrontal BOLD activation and correlated striatal dopamine release in heavy drinkers*. International Conference on Applications of Neuroimaging to Alcoholism, New Haven, CT, February 2013.

11. **DS Albrecht**, P MacKie, BT Christian, C Brown-Proctor, LM Federici, DA Kareken, CM Herring, JW Walters, KK Yoder. *Differential D<sub>2</sub>/D<sub>3</sub> receptor availability in fibromyalgia: associations with pain perception*. Society for Neuroscience 2012, New Orleans, LA September 2012.
12. **DS Albrecht**, P MacKie, BT Christian, C Brown-Proctor, LM Federici, DA Kareken, CM Herring, JW Walters, KK Yoder. *Initial assessment of the dopamine system in fibromyalgia*. 9<sup>th</sup> International Symposium on Functional Neuroreceptor Mapping of the Living Brain, Baltimore, MD August 2012.
13. KK Yoder, **DS Albrecht**, CM Herring, LM Federici, EA Patton, SJ O'Connor, DA Kareken. *Changes in striatal dopamine response to IV alcohol in nontreatment-seeking alcoholics but not social drinkers*. Accepted for oral presentation by K. Yoder, 9<sup>th</sup> International Symposium on Functional Neuroreceptor Mapping of the Living Brain, Baltimore, MD August 2012.
14. KK Yoder, **DS Albrecht**, CM Herring, DA Kareken. *Dopaminergic coding for alcohol-related negative prediction errors in alcoholics but not social drinkers*. 35<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Francisco, CA June 2012.
15. **DS Albrecht**, PD Skosnik, JM Vollmer, MS Brumbaugh, KM Perry, BH Mock, QH Zheng, LA Federici, EA Patton, CM Herring, KK Yoder. *Lower striatal D<sub>2</sub>/D<sub>3</sub> receptor availability in chronic cannabis users*. Society for Neuroscience 2011, Washington, D.C., November.
16. **DS Albrecht**, CM Herring, KM Perry, LM Federici, EA Patton, KK Yoder. *Effects of smoking on D<sub>2</sub>/D<sub>3</sub> striatal receptor availability in alcoholics and social drinkers*. 34<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, Atlanta, GA June 2011.
17. BG Oberlin, M Dzemidzic, **DS Albrecht**, KK Yoder, DA Kareken. *What combined fMRI and PET imaging of dopamine reveals about ventral striatal responses to tasting a favorite beer*. 34<sup>th</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, Atlanta, GA June 2011.
18. **DS Albrecht**, CM Herring, KM Perry, LM Federici, EA Patton, BH Mock, QH Zheng, KK Yoder. *Changes in striatal dopamine during negative prediction error in nontreatment-seeking alcoholics and social drinkers*. 33<sup>rd</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Antonio, TX June 2010.
19. KK Yoder, **DS Albrecht**, KM Perry, LM Federici, EA Patton, BH Mock, QH Zheng, CM Herring. *Striatal dopaminergic responses to IV alcohol in nontreatment-seeking alcoholics: a test-retest study*. 33<sup>rd</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Antonio, TX June 2010.
20. DA Kareken, MJ Walker, M Dzemidzic, V Bragulat, B Oberlin, **DS Albrecht**, KK Yoder. *Human ventral striatal dopamine receptor availability as a function of alcohol drink tastes*. ISBRA World Conference, Paris, France 2010.

21. **DS Albrecht**, MD Normandin, CA Cox, CM Herring, KP Perry, DG Garzon, DA Kareken, ED Morris, KK Yoder. *Effects of IV alcohol on dopamine release in smokers with a family history of alcoholism: Dependence on baseline D2 receptor binding availability*. 32<sup>nd</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Diego, CA June 2009.
22. KK Yoder, MD Normandin, CA Cox, CM Herring, KM Perry, DG Garzon, DA Kareken, ED Morris, **DS Albrecht**. *Reproducibility of a novel method for quantitating human dopamine release with [<sup>11</sup>C]raclopride: preliminary data from an alcohol challenge paradigm*. 32<sup>nd</sup> Annual Research Society on Alcoholism (RSA) Scientific Conference, San Diego, CA June 2009.
23. Karmen K. Yoder, MD Normandin, CA Cox, CM Herring, KM Perry, DG Garzon, DA Kareken, ED Morris, **DS Albrecht**. *Possible effects of family history of alcoholism on the test-retest variability of striatal D2 availability*. IXth International Conference on Quantification of Brain Function with PET. Chicago IL, USA June 29-July 3.
24. **DS Albrecht**, KK Yoder. *Effects of noisy PET data on within-subject measurement of striatal D2 receptor availability*. IUPUI Research Day, Indianapolis, IN April 2009.
25. **DS Albrecht**, HM Dam, JL Siegel, LA Porter. *Stability of Functionalized Porous Silicon in a Simulated Gastrointestinal Track*. Presented at:
  - American Chemical Society (ACS) National Meeting, Chicago, IL (2007)
  - American Chemical Society (ACS) Indiana Local Section Poster Session (2006)
  - Wabash Celebration of Student Research, Scholarship, and Creative Work (2007)

**AD-HOC REVIEWER:**

*Drug and Alcohol Dependence*

**RELATED EXPERIENCE:**

***Research:***

Lab Member, Yoder PET Research Laboratory, Department of Radiology, IUSM Indianapolis, IN, June 2008-present. (Mentor: Dr. Karmen Yoder, Ph.D.)

- PET data acquisition, image processing, quality control, and analysis (esp. [<sup>11</sup>C]raclopride, [<sup>18</sup>F]fallypride, [<sup>11</sup>C]CFT)
- Skin conductance recording
- Structured interviews of human subjects
- Intravenous alcohol infusion technique (“Indiana Alcohol Clamp”)
- Database management
- Administering and scoring neuropsychological testing battery to human subjects
- Manuscript preparation
- Certified for Human Subjects Research at the Indiana University School of Medicine.

- Certified for small animal research by Indiana University Animal Care and Use Committee
- Certified in Radiation Safety Procedures

Graduate Laboratory Rotation, Institute of Psychiatric Research, IUSM  
Indianapolis, IN, March-May 2008 PI: Zac Rodd

- Exposed rat pups to subcutaneous nicotine injections and oral alcohol intake to assess the effects of polydrug exposure during adolescence
- Learned stereotactic surgical techniques

Graduate Laboratory Rotation, Institute of Psychiatric Research, IUSM  
Indianapolis, IN, January-March 2008 PI: Nick Grahame

- Employed operant conditioning to assess impulsivity in high and low-alcohol preferring mice
- Handled animals daily and learned intraperitoneal injection technique

Graduate Laboratory Rotation, Stark Neurosciences Research Institute, IUSM  
Indianapolis, IN, October-December 2007 PI: Ted Cummins

- Learned cell culture techniques and the basics of site-directed mutagenesis and DNA sequencing
- Removed dorsal root ganglion cells from rat spinal cord and plated excised neurons

Research Assistant, Department of Chemistry, Wabash College  
Crawfordsville, IN, June-December 2006

- Created porous silicon chips using a hydrofluoric acid method
- Produced organic monolayers on chips via thermal, Lewis acid, and carbocation mediated pathways
- Tested chip stability in an artificial gastrointestinal environment
- Characterized chemical properties of silicon surface using Fourier transform infrared spectroscopy (FT-IR)

***Teaching:***

Chemistry Tutor, Department of Chemistry, Wabash College  
Crawfordsville, IN, 2006-2007

- Aided undergraduate chemistry students with coursework and lab reports.

**PROFESSIONAL DEVELOPMENT:**

***Courses:***

BME495 – Tracer Kinetics  
BME595 – Medical Imaging  
PSY 60000 – Statistical Inference

***Conferences:***

Annual Conference on Neuroeconomics: Decision Making and the Brain.  
September 2011, Evanston, IL.

***Training Sessions/Workshops:***

Professional Skills Training sponsored by 5T32AA007462-25, "Training Grant on Genetic Aspects of Alcoholism" (W. McBride, PI), led by Dr. C. Czachowski. Spring 2010.

"Write Winning Grants". Presented by Grant Writers' Seminars & Workshops. Stephen W. Russell and David C. Morrison. September 2010.

spInUp Fellow Program.

A short, intense course in technology commercialization, sponsored by the IURTC. April – May 2012.

Panelist for an educational panel on Mentorship sponsored by the Indiana University Office of Research Ethics, Education and Policy (REEP). 9/24/2013.