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The Dopamine D₂-like Receptor:
At the Nexus Between Self-Control and Addiction

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

by

Stephanie Mary Groman

2013

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ABSTRACT OF THE DISSERTATION

The Dopamine D₂-like receptor:

At the nexus between self-control and addiction

By

Stephanie Mary Groman

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2013

Professor James David Jentsch, Committee Chair

Addictions are multi-dimensional disorders, consisting of several behavioral, affective and cognitive dysfunctions that contribute to the compulsive and persistent drug-seeking and taking that is common to them. Cognitive control, which includes the ability to flexibly and adaptively inhibit undesirable actions (including drug-seeking), is a particularly relevant dimension of addiction, with deficits in cognitive control occurring in response to experience with drugs of abuse, as well as predicting the susceptibility for future drug-taking behaviors. The bi-directional relationship between cognitive control and substance dependence raises the possibility that these processes are governed by a common neural circuitry and emerging evidence indicates that the dopamine D₂-like receptor system may be the point of convergence of these phenomena.

To determine the influence of the dopamine D₂-like receptor system on cognitive control processes within the context of addictions, neuroimaging, behavioral and biochemical techniques were used to interrogate how naturally occurring and drug-induced variation in D₂-like receptor system may alter cognitive-control processes. Individual differences in D₂-like receptor availability, assessed with positron emission tomography, was positively related to adaptive responding following the reversal of

stimulus-reward contingencies and to the sensitivity of individuals to positive feedback. Exposure to an escalating dose regimen of methamphetamine reduced D₂-like receptor availability, and the degree of D₂-like receptor dysfunction was correlated with the change in positive-feedback sensitivity. Cross-dimensional measurement of the D₂-like receptor systems using *in vivo* and *in vitro* techniques provided evidence that deviations in D₂-like receptor availability reflected actions on functionally and behaviorally relevant pools of D₂-like receptors. Finally, evidence supporting the utility of spontaneous eye blink rate as a non-invasive measure of D₂-like receptors was obtained from studies of rodents.

These studies provide converging support, at multiple levels of analyses, that the D₂-like receptor is a common molecular determinant of addiction and cognitive control, providing a mechanistic explanation for the bi-directional relationship between these processes.

The dissertation of Stephanie Mary Groman is approved.

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2013

This dissertation is dedicated to my family and friends:

Your unwavering support has allowed me to achieve

things I never imagined possible

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Monoamine levels within the orbitofrontal cortex and putman interact to predict reversal learning performance. *Biological Psychiatry* [Epub ahead of print; doi: 10.1016/j.biopsych.2012.12.002]. Figures APP1.1-1.3 are reprinted with permission of Elsevier; Groman SM, Morales AM*, Lee B, London ED, and Jentsch JD (2013): Methamphetamine-induced increases in putamen gray matter associate with inhibitory control. *In press (Psych-2013-00153), Psychopharmacology*.

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CHAPTER 1

Background and Significance

Mental disorders are multi-dimensional syndromes, characterized by a collection of symptoms that often span conceptually unrelated behavioral and cognitive domains. Though classification of these symptoms is typically categorical, with the symptom being “present” or “absent” in an individual, and the symptoms “summing” to reach a syndromal category, a dimensional analysis of symptomatology may provide advantages in understanding the biological basis of mental disorders.

Symptom dimensionality may be a fundamental link for understanding inter-individual differences in response to treatment. Specifically, variation in symptom cluster and severity may explain why one intervention is highly beneficial for some individuals but with little to no therapeutic effect for others. There is often a lack of correlation between treatment strategies and psychosocial outcomes in individuals diagnosed with a variety of psychiatric disorders, such as schizophrenia, depression or addiction.

The development of effective and targeted treatments relies on advancements in basic research directed at elucidating the neurochemical abnormalities that underlie these disorders in order to develop or identify effective novel pharmacological agents. However, the translational of basic science results into effective pharmacotherapy has been fairly limited. This, too, may be a consequence of dichotomizing these disorders and symptoms, obfuscating the biological variability that exists within a disorder.

One potential strategy that has been proposed as a method to bridge the gap between basic scientific results, pharmacological development and therapeutic strategies is to deconstruct disorder-specific symptoms into simpler and more refined phenotypes. There are a numerous phenotypes that could be used to study specific dimensions of mental disorders (sensory-gating, feedback sensitivity, etc)

and aberrant cognitive control processes have been consistently proposed as core features of several psychiatric disorders.

The aim of the studies included in this dissertation is to provide evidence for the biological bases of cognitive control processes in the context of addictions. However, cognitive control represents an important dimension of many mental disorders and although the use of cognitive control to better understand addictions is the governing topic of this thesis, the same principles can be extended to other mental disorders including, but not limited to, anxiety, depression, and schizophrenia. In fact, deviations in cognitive control processes have been detected in individuals diagnosed with mood disorders, suggesting that cognitive control may be one important dimension of these disorders. Thus, investigating dimensions of disorders can further our neural understanding of psychopathologies and assist in developing scientifically-based, individualized-treatment strategies.

Phenotypic Overlap Between Addictions, Impulsivity and Cognitive Control

Substance dependence is defined as the compulsive, inflexible seeking and taking of drugs, despite the negative consequences associated with those behaviors. This concept likely extends to so-called “process addictions”, as there is evidence that similar forms of compulsive behaviors can develop in response to non-drug reinforcers, including food (Volkow et al., 2008a) and sex (Garcia and Thibaut, 2010). Irrespective of the goal that drives the addiction, the behavioral sequela of addictions are similar, suggesting that a common set of biological substrates contributes to this set of psychiatric phenotypes.

Key to our concepts of drug abuse and dependence are impulsive and compulsive patterns of drug seeking. For example, persistent use of a substance despite knowledge of the long-term, detrimental consequences may mirror the myopic characteristics of impulsivity (wherein immediate gratification outweighs delayed negative consequences). Further, reduced ability to voluntarily cease drug use can be viewed as a failure in the ability to exert inhibitory control over compulsive behaviors.

Indeed, several lines of evidence have implicated dimensions of impulsivity and cognitive control as core features of addictions (Jentsch and Taylor, 1999; Dawe and Loxton, 2004).

Impulsivity is a construct that describes a set of behaviors, including difficulty resisting urges, hasty or risk-prone decision-making and reduced sensitivity to delayed outcomes, which, in extreme forms, have the potential to be maladaptive (Winstanley et al., 2006). Many studies implicated aspects of impulsivity in addictions (Bickel et al., 1999; Alessi and Petry, 2003; Coffey et al., 2003; Monterosso et al., 2007; Weller et al., 2008). Specifically, higher levels of trait impulsiveness are observed in substance-dependent individuals (Madden et al., 1997; Mitchell, 1999; Coffey et al., 2003; Beck et al., 2009; Lee et al., 2009) or those who exhibit other types of addictions (Nasser et al., 2004; Fuentes et al., 2006).

Chronic exposure to drugs of abuse produces an enhancement in impulsive-like responding in animals (Richards et al., 1999; Dallery and Locey, 2005; Roesch et al., 2007), but high trait impulsivity also has been proposed as a risk factor for the development of substance dependence (Verdejo-Garcia et al., 2008; Esposito-Smythers et al., 2009; Ersche et al., 2010). Variability in impulsive-like behaviors in rodents can predict future self-administration of drugs (Perry et al., 2005; Dalley et al., 2007; Perry et al., 2008; Anker et al., 2009) and is associated with a punishment-resistant, drug-taking phenotype (Belin et al., 2008). Therefore, high impulsivity may be a consequence of substance use, as well as an indicator of susceptibility for developing substance dependence.

Individual differences in impulsive temperament are likely related to variation in cognitive control, which is defined as the ability to exert volitional control over one's thoughts, feelings and actions (Miller and Cohen, 2001). It is itself a multi-dimensional construct, involving several psychological processes and neural systems. Because cognitive control involves the utilization of representations of goals and/or abstract rules to guide behavior, it necessarily involves several higher-order processes, such as working memory, cognitive flexibility, response inhibition and goal-directed attention. Relatively poor function in any of these domains can contribute to inflexibility of behaviors

and/or mental states and consequently may underlie dimensions of a number of psychiatric disorders, including (but not limited to) addictions (Kubler et al., 2005; Fillmore and Rush, 2006; Ersche et al., 2008; Indlekofer et al., 2009; Kalechstein et al., 2010). Indeed, there is growing evidence that impulsivity and cognitive control are directly related (Groman et al., 2007; James et al., 2007; Romer et al., 2009), suggesting that impulsivity may represent a higher-order phenotypic consequence of extreme deficits in multiple dimensions of cognitive control.

As predicted, cognitive control deficits have been observed in individuals dependent upon a variety of substances (Kubler et al., 2005; Fillmore and Rush, 2006; Ersche et al., 2008; Indlekofer et al., 2009; Kalechstein et al., 2010), as well as in animals chronically exposed to drugs of abuse (Jentsch et al., 2002; Crean et al., 2010; Porter et al., 2011), indicating that the materialization of these deficits may, in part, be due to chronic drug use/exposure. However, deficits in cognitive control processes that precede drug use may themselves influence the development of dependence. Children who are at high risk for the development of substance abuse, based upon familial patterns of alcoholism (Deckel and Hesselbrock, 1996), have purported cognitive control impairments prior to any drug use (Giancola et al., 1996b; Aytaclar et al., 1999). Further, variation in cognitive control processes correlates with behavioral indicators of substance abuse problems, such as severity of drug use and quantity of drug experimentation (Aytaclar et al., 1999), and preclinical predictors of drug reinforcement (Dellu-Hagedorn, 2005).

Although cognitive control deficits are not part of the current diagnostic criteria for addictions or other psychiatric disorder, they have been proposed to be defining characteristics of addictions and to be both an indicator of susceptibility to the condition and a potential behavioral target for the treatment of substance dependence (Jentsch and Taylor, 1999; Klingberg et al., 2002; Groman et al., 2009). Indeed, pharmacological treatments that enhance cognitive control have also been reported to reduce symptoms in individuals affected by addictions (Dackis et al., 2005; Anderson et al., 2009;

Shearer et al., 2009), and performance on tasks of cognitive control is correlated with predictors of sobriety (Moeller et al., 2001; Bowden-Jones et al., 2005; Aharonovich et al., 2006), suggesting that impulsivity and cognitive control are important dimensions that directly influence the ability of individuals to cease substance use (Jentsch and Taylor, 1999).

The high degree of overlap between cognitive control, impulsivity and behavior addictions suggest that these processes are governed by similar, overlapping mechanisms. Indeed, there is substantial evidence that the dopaminergic system within the corticostriatal circuit is a point of convergence for these behavioral and cognitive processes.

The Corticostriatal Circuit As A Common Neural Pathway

Anatomical and biochemical studies examining the anatomical basis of cognitive control, impulsivity and behavioral addictions have, independently and convergently, have identified the brain nuclei within the corticostriatal circuit as critical brain regions of these phenotypes. The corticostriatal circuit is composed of a series of segregated loops between cortical, striatal and midbrain structures that are topographically organized: limbic and associative information arising from the prefrontal cortex innervates the medial portion of the striatum, whereas sensory and motor information from the premotor and motor cortex innervate the lateral portion of the dorsal striatum (Haber, 2003). The topographical organization is retained in the striatal projections to the pallidum and substantia nigra, which finally relay back to the cortex, via the thalamus (Redgrave et al., 2010). Because of these parallel, looping projections, neural signals embedded in this circuit are susceptible to modulation at any of these points and alterations, biochemically and anatomically, in any of these brain regions influence the circuit, and therefore the signal, as a whole.

Anatomical, biochemical and functional alterations within the corticostriatal circuitry have been implicated in both substance and process addictions. Specifically, prefrontal gray-matter volume and density are lower in substance dependent individuals (Liu et al., 1998; Fein et al., 2002; Franklin et al., 2002; Thompson et al., 2004; Jang et al., 2007; Liu et al., 2009; Tanabe et al., 2009; Schwartz et al., 2010; Yuan et al., 2010) and morbidly-obese individuals (Raji et al., 2010; Maayan et al., 2011), compared to that of healthy comparison subjects. Functional and metabolic studies have also reported reduced connectivity between prefrontal and subcortical structures (Lim et al., 2002; Chung et al., 2007), and altered glucose metabolism in prefrontal (Volkow et al., 1993; London et al., 2004; Kim et al., 2005; Kim et al., 2009) and striatal regions (London et al., 2004; Sevy et al., 2008), in substance dependent individuals. Similar abnormalities in morphology and glucose metabolism have been reported in animals exposed to drugs (Hammer et al., 1993; Robinson and Kolb, 1997; Robinson et al., 2001; Porrino et al., 2004; Crombag et al., 2005), implicating drug use as the mechanism by which the neural alterations observed in substance-dependent individuals manifest.

Although no studies to date have directly examined whether variation in corticostriatal integrity is predictive of future addictions, reduced gray matter in cortical and sub-cortical brain nuclei is present in alcohol-naïve adolescents at high risk for substance dependence (Benegal et al., 2007). Additionally, animal studies have provided evidence that experimentally-induced damage to the prefrontal cortex, prior to drug exposure, enhances the acquisition and performance of drug self-administration (Weissenborn et al., 1997). Therefore, pre-existing variation in corticostriatal integrity may directly influence drug reinforcement, which may eventually develop into substance dependence.

Similarly, brain regions within the corticostriatal circuit have been implicated in impulsivity and impulsive-like behaviors: individuals with damage to the ventral and orbital frontal regions consistently report higher levels of impulsivity (Bechara et al., 2000; Berlin et al., 2004), with analogous results being observed in animals with lesions to the prefrontal cortex (Chudasama et al., 2003) and striatal regions

(Cardinal et al., 2001; Rogers et al., 2001). Furthermore, relatively small deviations in corticostriatal integrity have been reported to be associated with impulsivity. Specifically, gray-matter density within the prefrontal cortex and ventral striatum is correlated with self-reported levels of impulsivity (Carmona et al., 2009; Matsuo et al., 2009) and delay discounting functions (Bjork et al., 2009) in otherwise healthy individuals, implicating disruptions in the corticostriatal circuit as an anatomical determinant of impulsivity.

The prefrontal cortex has been well established as being essential for several dimensions of cognitive control, as damage to this region is associated with impairments in response inhibition (Iversen and Mishkin, 1970; Aron et al., 2003; Szatkowska et al., 2007; Swick et al., 2008), working memory (Funahashi et al., 1993; Manes et al., 2002; du Boisgueheneuc et al., 2006), attention (Godefroy and Rousseaux, 1996) and behavioral/cognitive flexibility (Dias et al., 1996a; Dias and Aggleton, 2000; Hornak et al., 2004; Rygula et al., 2010). However, similar deficits have been reported in animals following lesions to the striatum (Floresco et al., 1997; Rogers et al., 2001; Clarke et al., 2008; Castane et al., 2010), indicating that these processes depend upon the coordinated activity of a linked corticostriatal network. Indeed, functional imaging studies have reported activation of several corticostriatal-brain nuclei during tasks of working memory (Levy et al., 1997) and behavioral flexibility (Xue et al., 2008; Ghahremani et al., 2010) providing evidence that these processes rely upon a large network of regions in the corticostriatal circuit.

Deficits in cognitive control processes are not unique to addictions. Individuals diagnosed with mood disorders, such as depression, bipolar disorder or anxiety, have deficits in several domains of cognitive control (Taylor Tavares et al., 2008; McKirdy et al., 2009; Dickstein et al., 2010; Maalouf et al., 2010) and abnormalities in corticostriatal-related nuclei (Ballmaier et al., 2004; van Tol et al., 2010; Chang et al., 2011; Foland-Ross et al., 2011; Houenou et al., 2011). Therefore, abnormalities in structure and/or function of the corticostriatal circuit may be the mechanism by which phenotypic variation in

cognitive processes, such as response inhibition or behavioral flexibility, arise in a number of psychiatric disorders.

Dopamine as the Common Neural Substrate

Brain nuclei of the corticostriatal circuit receive dopaminergic innervation from midbrain dopamine (DA) neurons. Specifically, the prefrontal cortex receives direct dopaminergic projections from mesencephalic dopaminergic neurons and projects back to DA and GABAergic interneurons within the midbrain (Carr and Sesack, 2000). In addition to this direct feedback pathway, the prefrontal cortex also sends excitatory projections to the striatum, enabling direct control over midbrain-mediated DA release in the ventral and dorsal striatum (Karreman and Moghaddam, 1996). Depletion of DA content in the prefrontal cortex increases extracellular DA levels in the basal ganglia (Pycock et al., 1980; Martin-Iverson et al., 1986; Deutch et al., 1990; Carlson et al., 1996) and DA release in response to reinforcing stimuli (Mitchell and Gratton, 1992), demonstrating the involvement of prefrontal DA in modulating baseline and stimulus-elicited striatal DA efflux. These neural systems are thought to contribute to top-down control of the prefrontal cortex over subcortical DA projections, and dysfunction within any one of these brain regions alters dopaminergic tone in the nuclei that comprise the corticostriatal circuit (Roberts et al., 1994; Karreman and Moghaddam, 1996). Because of the relationships between corticostriatal-related nuclei and cognitive control, impulsivity, and behavioral addictions, dopaminergic dysfunction within this circuitry is believed to underlie these phenotypes.

Despite the diverse pharmacological targets of stimuli with reinforcing and/or rewarding properties, all increase extracellular dopamine levels within the ventral striatum (Di Chiara and Imperato, 1988; Pfaus et al., 1995; Pontieri et al., 1995; Bassareo and Di Chiara, 1997). Therefore, DA is believed to be involved in the incentive value and motivational properties of rewards (Berridge and Robinson, 1998) and is implicated in the neural circuitry of disorders involving abnormal reward-seeking

and -taking. While acute administration of drugs with abuse liability increases striatal DA tone, lower levels of dopamine have been found in post-mortem brain tissue of cocaine- and heroin-dependent individuals (Wilson et al., 1992; Kish et al., 2001; Little et al., 2009), as well as in animals chronically exposed to drugs (Segal and Kuczenski, 1992b, a; Maisonneuve et al., 1995; Sorg et al., 1997; Henry and Howell, 2009; Lee et al., 2011a). Studies utilizing *in vivo* imaging techniques have found similar dopaminergic alterations, specifically with lower levels of DA, rates of DA synthesis and drug-induced DA release being observed in cocaine- (Volkow et al., 1997; Martinez et al., 2007; Martinez et al., 2009) and alcohol-dependent individuals (Heinz et al., 2005; Volkow et al., 2007a).

Although a hypodopaminergic striatal system may be a consequence of chronic drug use (Melega et al., 2008; Lee et al., 2011a), low DA tone predating drug use may directly influence drug taking and the progression of dependence. ADHD patients, who are at a substantially greater risk for developing substance dependence (Biederman et al., 1995; Lee et al., 2011b), have lower drug-induced striatal dopamine release (Rosa-Neto et al., 2005; Volkow et al., 2007b). These findings parallel earlier work providing causal evidence for this relationship in high alcohol preferring rats (Gongwer et al., 1989; George et al., 1995; Quintanilla et al., 2007). Therefore, low DA signaling may drive individuals to seek out and obtain rewards that increase DA levels as a way to compensate for their pre-existing hypodopaminergic state.

Several recent studies have demonstrated that variation in the DA system underlies impulsivity, such that individuals who report greater levels of impulsiveness have greater amphetamine-induced DA release in the ventral striatum (Buckholtz et al., 2010b). Further, administration of the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA), can induce impulsive responding in healthy subjects (Pine et al., 2010) and in Parkinson's disease patients (Cools et al., 2003). Preclinical evidence indicates that impulsivity may be, in part, be mediated by dopamine's actions in the striatum: amphetamine-induced increases in impulsive-like responding are attenuated following focal DA depletion within

ventral and dorsal striatal regions (Cole and Robbins, 1989; Baunez and Robbins, 1999). Thus, the enhanced DA release in the striatum in high-impulsive individuals may be a neurochemical consequence of prefrontally-mediated dysfunction of control over DA release within the striatum.

The DA system has been broadly implicated in modulating cognitive control processes. Targeted DA depletion of the prefrontal cortex and striatum impair performance in several tasks of cognitive control (Brozoski et al., 1979; Roberts et al., 1994; Collins et al., 2000; Crofts et al., 2001; O'Neill and Brown, 2007; Clarke et al., 2011), and variation in striatal dopamine synthesis is correlated with performance on tasks of working memory and behavioral flexibility (Vernaleken et al., 2007; Cools et al., 2008; Cools et al.; Landau et al., 2009). Although several linear, monotonic relationships between DA and cognitive control have been reported, greater DA tone in prefrontal and striatal regions can also produce cognitive control deficits similar to those associated with low DA tone (Arnsten et al., 1994; Murphy et al., 1996; Cai and Arnsten, 1997), indicating that the relationship between DA levels and cognitive control may be non-linear (Arnsten et al., 1994; Cai and Arnsten, 1997; Cools et al., 2009). Therefore, deviations in dopaminergic tone within the corticostriatal circuitry may explain individual variation in cognitive control processes amongst both clinical and non-clinical populations (Cools et al., 2008).

Although a hypodopaminergic state may drive individuals to seek out and/or obtain rewards that neurochemically elevate DA levels, cognitive control may directly modulate this relationship. Rigid or inflexible behaviors present prior to drug use may influence the developmental time course of addictions, predisposing individuals to develop habitual, compulsive behaviors at a rate much faster than those individuals with normal or high cognitive control function. This may be due, specifically, to dopamine's influence on both of these processes, whereby low dopaminergic tone results in an enhanced drive to obtain rewards as well as impairing the ability to exert egocentric control over the very same behaviors that are reinforced by the persistent use of rewards.

The DA D₂/D₃ Receptor System as a Common Biochemical Mechanism

The functional effects of DA are mediated by two classes of metabotropic receptors known as the D₁-like and D₂-like families. D₁-like receptors, comprised of the D₁ and D₅ receptor subtypes, are G_s coupled and, when activated, increase adenylate cyclase levels. D₂-like receptors, comprised of the D₂, D₃ and D₄ receptor subtypes, are G_i coupled and when stimulated, formation of adenylate cyclase is either decreased or is unaltered (Iversen et al., 2008). Both D₁-like and D₂-like receptors are expressed on post-synaptic terminals in brain nuclei that receive dopaminergic input; however, D_{2/3} receptors are also found pre-synaptically where they act as autoreceptors, regulating both DA release and synthesis. Because D₂-like receptors convey the DA signal post-synaptically as well as regulating overall dopaminergic tone, alterations to this receptor can have both profound, as well as variable, effects on the signaling profile of DA. Therefore, the D₂-like receptor is of particular interest in disorders, such as but not limited to addictions, that are believed to be a result of dopaminergic dysfunction.

D₂-like receptor availability has been consistently reported as being lower in individuals dependent on a variety of substances, such as cocaine (Volkow et al., 1993), methamphetamine (Volkow et al., 2001b; Lee et al., 2009), nicotine (Fehr et al., 2008), opiates (Zijlstra et al., 2008), and alcohol (Tupala et al., 2001; Tupala et al.; Martinez et al., 2005), as well as in morbidly-obese individuals (Volkow et al., 2008b). The alterations in D₂-like receptor levels appear, in part, to be mediated by chronic exposure to rewards, as similar reductions in D₂-like receptor levels have been reported in animals following chronic exposure to cocaine (Nader et al., 2006), alcohol (Thanos et al.; Thanos et al., 2004) and high caloric food (Johnson and Kenny, 2010).

However, animals with pre-existing low levels of the D₂-like receptor self-administer cocaine at greater rates than those with higher D₂-like receptors (Nader et al., 2006; Dalley et al., 2007), and viral vector-mediated knock down of D₂ receptors has produced compulsive eating in mice (Johnson and Kenny, 2010). Based upon this evidence, low levels of the D₂-like receptor have been proposed to be a

consequence as well as a risk factor for substance use, and pharmacological manipulations that increase D₂-like receptor function have been proposed as a possible treatment for substance dependence. Indeed, there is evidence that increasing levels of D₂-like receptors attenuates alcohol consumption (Thanos et al., 2004) and cocaine self-administration (Thanos et al., 2008) and that above-normal levels of the D₂-like receptor may act as neuroprotective factor for individuals with familial alcoholism (Volkow et al., 2006).

Recent evidence has highlighted the involvement of the D₂-like receptor system in impulsivity and impulsive-like behaviors. D₂-like receptor availability in the striatum (Lee et al., 2009) and midbrain (Buckholtz et al., 2010b) negatively correlate with self-report levels of impulsiveness. Genetic studies examining the DRD2 gene have found that individuals that carry the TaqIA allele, a variant associated with reduced striatal D₂-like receptor availability (Thompson et al., 1997), report higher levels of impulsivity (Colzato et al., 2010) and exhibit steeper discounting of delayed rewards (Eisenberg et al., 2007).

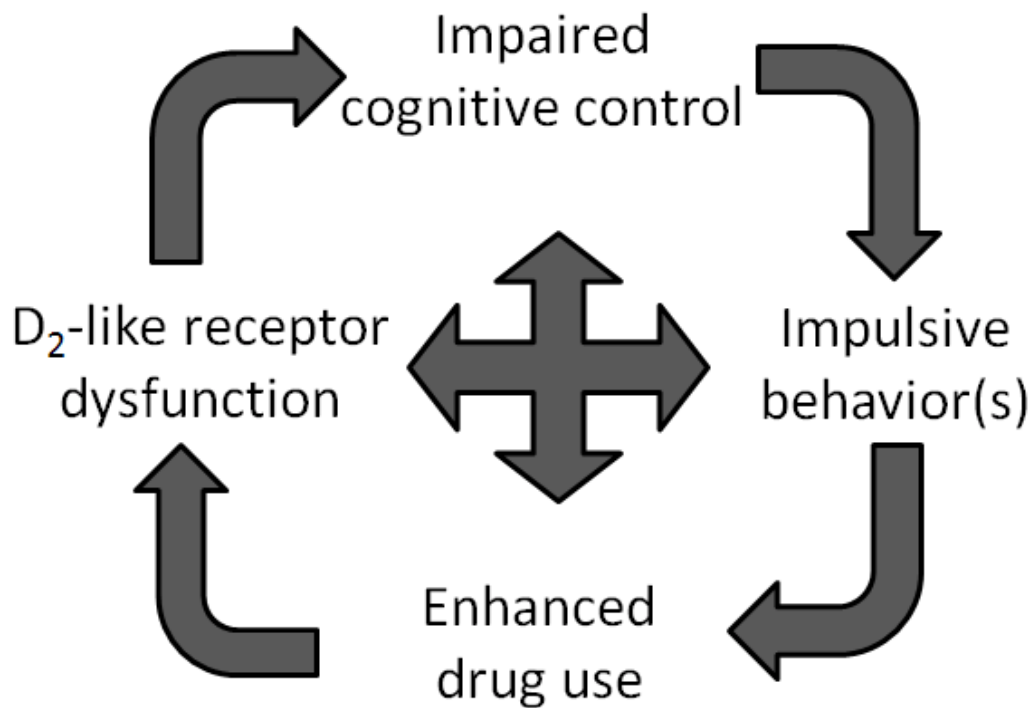
The DA D₂-like receptor system is involved in several cognitive processes, most notably behavioral and/or cognitive flexibility (Floresco et al., 2006; Lee et al., 2007; Boulougouris et al., 2009; Groman et al., 2011; Laughlin et al., 2011) and working memory (Luciana et al., 1992; Mehta et al., 2004; Bertolino et al., 2009). Pharmacological blockade of D₂-like receptors impairs reversal-learning performance (Lee et al., 2007; Herold, 2010) and working memory (Mehta et al., 2004), with similar results being found in mice lacking the D₂ receptor gene (Glickstein et al., 2005; De Steno and Schmauss, 2009) and carriers of the DRD2 TaqIA allele (Jocham et al., 2009). Although the mechanism by which the D₂-like receptor system modulates cognitive control processes is unknown, striatal D₂-like receptor availability is positively correlated with glucose metabolism in prefrontal regions (Volkow et al., 1997). Therefore, improving DA D₂-mediated transmission may directly modulate prefrontal dependent activity, improving aspects of cognitive control.

A recent study has provided evidence that ties these behavioral and biochemical processes together, demonstrating that administration of a D₂-like receptor agonist improves reversal-learning deficits in stimulant dependent individuals (Ersche et al., In press). Therefore, the cognitive control impairments present in individuals with an addiction may be a behavioral manifestation of abnormal D₂-mediated dopamine transmission.

D₂-like Receptor Dysfunction: A Common Substrate for Cognitive Control, Impulsivity and Substance Dependence

Dysfunction of the D₂-like receptor system represents a common biochemical mechanism underlying cognitive control, impulsivity and addictions. Based upon the presented evidence, we propose that reductions in D₂-like receptor function contribute to the development of addictions through two primary mechanisms that are inter-related (see Figure 1).

First, low D₂-like receptor function prior to drug use results in aberrant positive feedback processing (Groman et al., 2011), resulting in inflexible, habitual behaviors. Impairments in the ability of individuals to exert control over their behaviors manifests as heightened levels of impulsivity that promote compulsive consumption of rewards (Dalley et al., 2007; Belin et al., 2008), and eventually, as reward use continues, can lead to the development of dependence. Second, chronic intake of rewards can reduce D₂-like receptor function (Nader et al., 2006; Johnson and Kenny, 2010) resulting in aberrant positive feedback integration which promotes the rapid development of habitual behaviors and heightened levels of impulsivity that enhances substance use and, eventually, the development of dependence.



Although convergent lines of evidence, stemming from a variety of techniques used in human and animal subjects, support this hypothesis, several questions remain unaddressed. First, although pharmacological evidence indicates a selective role of D₂-like receptors in cognitive control, it is unknown whether individual differences in D₂-like receptor availability, due to genetic or environmental factors, relate directly to cognitive control. Additionally, the decrements in cognitive control function and D₂-like receptor availability detected in animals chronically exposed to drugs have not been concurrently measured within the same subjects, limiting the ability to draw causal inferences between changes in D₂-like receptor availability and cognitive control. Finally, the biological relevance of D₂-like receptor dysfunction detected in substance dependent individuals remains unaddressed, given the possibility that they may reflect changes in receptor populations that have no functional impact on the behaviors underlying substance dependence, rendering them irrelevant targets for the treatment of addiction. Therefore, studies integrating multiple techniques that span diverse levels of analyses to

address these limitations can provide the necessary evidence that the D₂-like receptor systems is at nexus between self control and addiction.

Overview of Current Project

The aim of this dissertation is to elucidate the associations between the biochemical and behavioral disruptions that have been observed in substance dependent individuals. Specifically, it examines the hypothesis that pre-existing and drug-induced dysfunction of the D₂-like receptor system is the molecular determinant by which cognitive control deficits manifest in substance-dependent individuals. By combining neuroimaging assessments of D₂-like receptors with behavioral measures of cognitive control before and after exposure to methamphetamine, the first two aims of this dissertation will provide insight into the behavioral relevance of D₂-like receptor dysfunction before and after drug exposure. The third aim will provide the framework needed for the interpretation of neuroimaging results of D₂-like receptors, utilizing PET to assess dopamine receptor availability, behavior, pharmacology, and biochemical techniques to determine how the functional state of D₂-like receptors co-varies with neuroimaging measurements. Finally, the last aim is directed at providing evidence for the utility of spontaneous eye blink rate as proxy of D₂-like receptor function in rodents.

Aim 1: Linking D₂-like receptor availability with inhibitory control in a healthy population of monkeys

Previous studies, using pharmacology, have indicated that the D₂-like receptor system is selective involved in the ability of subjects to reverse a stimulus-reward association; however, it remains to be known whether naturally occurring differences in D₂-like receptor availability, most likely under genetic control, co-vary selectively with inhibitory control. To address this, positron emission tomography (PET) will be used to measure individual differences in D₂-like receptor availability which will be related to the behavioral performance of monkeys trained to acquire, retain and reverse novel, visual discrimination problems. The results of this study will allow us to determine how naturally

occurring variation in D₂-like receptor availability within distinct brain nuclei co-varies with a range of behavioral processes.

Aim 2: Investigating the effects of methamphetamine exposure on the D₂-like receptor and inhibitory control

Although previous studies, in animals, have demonstrated that chronic-cocaine exposure reduces D₂-like receptor availability in a manner similar to those detected in cocaine-dependent individuals, there is little evidence supporting this causal mechanism for the low D₂-like receptor availability observed in methamphetamine-dependent individuals and whether these changes in D₂-like receptors have a functional impact on measures of cognitive control. The second aim will attempt to provide a mechanistic link between drug-induced D₂-like receptor alterations and inhibitory control impairments by measuring cognitive control processes and D₂-like receptor availability before and after monkeys are exposed to a dosing regimen of methamphetamine that is similar to human patterns of methamphetamine intake. This study will allow us to determine if methamphetamine-induced changes in D₂-like receptor track with changes in cognitive control, providing a mechanistic link between the biochemical and behavioral impairments observed in substance-dependent populations.

Aim 3: Determine the functional and behavioral relevance of variation in D₂-like receptor availability

The results of the previous studies (Aim 1 and Aim 2) indicate that variation in D₂-like receptor availability is associated with cognitive control. However, *in vivo* neuroimaging techniques, such as PET, do not resolve whether these changes reflect changes in the density of these receptors, as availability measurements assessed with neuroimaging can be influenced by multiple changes in receptor state and function. To determine the precise manner in which differences in D₂-like receptor availability emerge, this study combines neuroimaging, pharmacology, biochemistry and behavioral techniques within the same subjects to ascertain the functionality of receptors and density of striatal D₂-like receptors. These results will provide a framework for interpreting PET measurements of D₂-like receptors.

Aim 4: Cross-species evidence for the utility of eye blink rate as a proxy of D₂-like receptor function

The results gathered in Aim 3 indicated that spontaneous eye blink rate may serve as a proxy of D₂-like receptor density and function. However, the translational applicability of this relationship in other species is unknown and additional studies using causal manipulations are needed to support our previous finding. To do such, eye blink rate was measured in rodents before and after exposure to a 30 d regimen of haloperidol (or vehicle), which has been previously shown to increase striatal D₂-like receptor density robustly. The results of this study will provide further support that spontaneous eye blink rate may serve as a proxy of D₂-like receptors, providing a non-invasive technique capable of addressing scientific questions that cannot be done with the currently available techniques.

Chapter 2

Dorsal Striatal D₂-Like Receptor Availability Co-varies with Sensitivity to Positive Reinforcement during Discrimination Learning

Summary

Deviations in reward sensitivity and behavioral flexibility, particularly in the ability to change or stop behaviors in response to changing environmental contingencies, are important phenotypic dimensions of several neuropsychiatric disorders. Neuroimaging evidence suggests that variation in dopamine signaling through dopamine D₂-like receptors may influence these phenotypes and associated psychiatric conditions, but the specific neurocognitive mechanisms through which this influence is exerted are unknown. To address this question, we examined the relationship between behavioral sensitivity to reinforcement during discrimination learning and D₂-like receptor availability in vervet monkeys. Monkeys were assessed for their ability to acquire, retain and reverse three-choice, visual-discrimination problems, and once behavioral performance had stabilized, they were subjected to positron emission tomography (PET) scans. D₂-like receptor availability in dorsal aspects of the striatum was not related to individual differences in the ability to acquire or retain visual discriminations but did relate to the number of trials required to reach criterion in the reversal phase of the task. D₂-like receptor availability was also strongly correlated with behavioral sensitivity to positive, but not negative, feedback during learning. These results go beyond electrophysiological findings by demonstrating the involvement of a striatal dopaminergic marker in feedback sensitivity and behavioral flexibility, providing insight into the neural mechanisms that are affected in neuropsychiatric disorders that feature these deficits.

Introduction

Impaired ability to update behaviors and actions rapidly in response to changes in environmental rules is exhibited by individuals with externalizing and impulsive-control disorders, and this dysfunction may be related to deviations in behavioral sensitivity to reinforcement, to poor inhibitory control, or to both (Jentsch and Taylor, 1999; Johansen et al., 2009). Because behavioral inflexibility may represent heritable factors that index risk for ADHD and addictions (Jentsch and Taylor, 1999; Groman et al., 2009; Ersche et al., 2010), understanding the biological mechanisms that mediate individual differences could illuminate the basis of these neuropsychiatric disorders.

Sensitivity to reinforcing feedback and behavioral flexibility can be studied objectively by examining the ability to acquire and reverse discrimination problems. In these tasks, subjects select from an array of stimuli, each being associated with availability or absence of positive reinforcement. Subjects progressively learn to direct their behavior to the stimuli associated with desirable outcomes. After achieving competency in the initial acquisition stage, the contingencies of the task are reversed, requiring that the subjects adapt their behavior. Both initial discrimination acquisition and reversal learning require sensitivity to reinforcement feedback, but the reversal-learning stage also involves a change from an established response pattern.

The ability to update behavior in response to rule reversal has been associated with integrity of the orbitofrontal cortex (McEnaney and Butter, 1969; Dias et al., 1996b) and dorsomedial striatum (Clarke et al., 2008; Castane et al., 2010). This corticostriatal circuit is modulated by dopamine, which may act subcortically (O'Neill and Brown, 2007; Cools et al., 2009). Pharmacological studies have shown a specific involvement of the D₂/D₃ (D₂-like) receptor system in reversal-learning performance across species (Lee et al., 2007; Boulougouris et al., 2009; Cools et al., 2009; Herold, 2010). Reversal-learning deficits are also observed in human carriers of the A1 allele of the Taq1a polymorphism in the D₂ receptor gene (Jocham et al., 2009), a variant associated with lower striatal D₂-like receptor availability

(Pohjalainen et al., 1998). Furthermore, pharmacological perturbations to the D₂-like receptor system influence sensitivity to feedback, such that D₂-like receptor agonists facilitate adjustments in behavior elicited by positive feedback, while D₂-like antagonists have the opposite effect (Frank and O'Reilly, 2006). Moreover, Taq1 A1 allele carriers exhibit impaired learning in response to negative feedback (Frank and Hutchison, 2009), suggesting that having relatively low levels of D₂-like receptors may confer behavioral inflexibility by altering feedback sensitivity.

To examine the question of how naturally occurring variation in sensitivity to feedback during reversal learning relates directly to D₂-like receptor density, we combined assessments of responding during acquisition, retention and reversal of discrimination problems with positron emission tomographic (PET) measures of D₂-like receptor availability in non-human primates. On the basis of the available data, we hypothesized that D₂-like receptor availability in the striatum would be correlated with negative-feedback sensitivity (Frank and Hutchison, 2009) and that this relationship would be exaggerated under reversal-learning conditions, when the demands to use feedback to guide behavior are greatest.

Methods

Subjects: Twelve male vervet monkeys (*Chlorocebus aethiops sabaesus* from the UCLA Vervet Research Colony), ranging from 5 to 9 years of age, were included in this study. Monkeys were individually housed in a climate-controlled vivarium, where they had unlimited access to water and received twice-daily portions of standard monkey chow (Teklad, Madison, WI). All of the subjects were able to see, hear and communicate with other individuals in the room. Monkeys received half of their daily portion of allotted chow in the morning after behavioral testing was conducted (approximately 1100 h) and their second half in the afternoon (approximately 1500 h); the total amount of chow received was never reduced during the experiment to facilitate task performance.

All monkeys were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Discrimination Acquisition, Retention and Reversal Learning: Monkeys were trained to move from their individual cages into a transport cart, and were brought to a quiet testing room where the transport cart was aligned to a Wisconsin General Testing Apparatus, which has been described elsewhere (Lee et al., 2007). It was equipped with an operable opaque screen that separated the monkeys from three equally spaced opaque boxes. Each box was equipped with a hinged opaque lid so that food rewards (small piece of apple, banana, grape or orange) could be concealed inside. Moreover, each box lid could be fitted with a unique visual stimulus (clip art from the Microsoft Office® library that consisted of colored objects unfamiliar to the monkey) that the monkeys could easily view when sitting at the apparatus.

Testing sessions began when the opaque screen was raised to present the three boxes (each fitted with a unique stimulus) to the monkey. Only one response, in which the monkey opened a box

fitted with a stimulus, was allowed per trial. A trial ended after a correct choice, an incorrect choice or an omission (no response for 2 min), and a 20-s intertrial interval followed. The next trial ensued with a different spatial box sequence, but with the reward associated with the same visual stimulus. Up to 80 trials per session were conducted.

Monkeys were trained to acquire, retain and reverse novel visual discriminations. The first session of a discrimination problem was a discrimination-acquisition phase and was held on a Monday or Thursday. The monkey was presented with three novel stimuli and had to learn which one was associated with reward, solely on the basis of trial and error. After a performance criterion (seven correct choices within ten consecutive trials) was reached, the session was terminated and the monkey was returned to his home cage. If a monkey did not reach criterion within 80 trials, the session ended but the same discrimination problem was presented the following day(s) until the performance criterion was met.

One day after reaching criterion, subjects were assessed in the retention phase, during which stimulus-reward contingencies were unchanged, until a criterion of four correct choices in five consecutive trials was met. The reversal phase then began immediately with no explicit signal that the transition between retention and reversal had occurred, other than the change in feedback experienced by the subject. During the reversal phase, the stimulus that was previously rewarded was no longer rewarded, and one of the two previously non-rewarded stimuli was rewarded. The reversal phase continued until the monkey achieved criterion (seven correct choices in ten consecutive trials) or until 80 trials had been completed, whichever occurred first. The number of trials required to reach criterion in the acquisition, retention and reversal phases were the primary dependent measures. For the reversal phase, the number of responses directed at the previously rewarded stimulus (perseverative responses) and the number of responses directed at the never rewarded stimulus (neutral responses) were also

measured. The probability of a monkey making each response type was also calculated by dividing the number of correct, perseverative or neutral responses by the total number of trials in the reversal phase.

Subjects acquired and reversed consecutive discrimination problems, each of which featured three novel visual stimuli. Due to technical delays in the acquisition of PET scans, the total number of discrimination problems completed and the number of days between completion of the last discrimination problem and the PET scans differed and are exhibited in Table 1; therefore, the analysis described here focused on the averages of the dependent measures collected across the last three problems, as these were closest in time to the subsequent PET scans.

Feedback Sensitivity Measures: Because behavioral sensitivity to positive and/or negative feedback can affect learning performance, we examined choice behavior on a trial-by-trial basis during the reversal phase. Here, we categorized trials according to whether the subject experienced positive or negative feedback on the preceding trial. This allowed calculation of the probability that after experiencing positive feedback, a subject would make either: a) another correct response, b) a response directed to the stimulus that was previously rewarded or c) a response directed at the stimulus that was never rewarded. The response to negative feedback was assessed by calculating the probability that a negative feedback event would be followed with either: a) the same incorrect response or b) a response directed at a different stimulus, irrespective of whether this response was correct or incorrect. We also performed a similar analysis of choice behavior for the data gathered during the discrimination acquisition; however, because perseverative responses were not possible in this phase, behavioral sensitivity to positive feedback was calculated by examining the probability of following a correct response with either: a) another correct response or b) an incorrect response.

[¹⁸F]fallypride/PET Scans: A variable number of days after behavioral performance had stabilized (Table 1), D₂-like receptor availability was assessed using a microPET Model P4 scanner (Concorde Instruments, Knoxville, TN). Dopamine transporter (DAT) availability was assessed, using [¹¹C]WIN-35,428, in the same subjects for a larger study; however, DAT availability measures were not included in the hypothesized mechanism for our primary analyses and, therefore, are not described here. Monkeys received an intramuscular injection of ketamine hydrochloride (10 mg/kg) and glycopyrrolate (0.01 mg/kg). After monkeys were immobilized, an endotracheal tube was placed to provide inhalation of 2-3% isoflurane (in 100% O₂) anesthesia throughout the duration of the experiment. Vital signs (heart rate, respiratory rate, oxygen saturation and temperature) were monitored and recorded every 15 min throughout the scan. A tail-vein catheter was placed, and the monkey was positioned on the scanning bed such that the imaging planes were parallel to the orbitomeatal line and the top of the head was at the front of the field of view. A 20-minute ⁶⁸Ge transmission scan was acquired before administration of the radioligand for attenuation correction. All subjects received a bolus injection [¹¹C]WIN 35428 (1.0 mCi/kg) , followed by a 5-mL saline flush, and data were acquired for 90 min. When radioactivity had fallen to baseline levels (~3 h after [¹¹C]WIN-35,428 administration), a bolus injection of [¹⁸F]fallypride was delivered (0.3 mCi/kg), followed by a 5-mL saline flush. Dynamic data were acquired in list mode for 180 min. After the scan, animals were removed from the gas anesthesia and allowed to recover overnight before being returned to their home cages.

Reconstruction of PET images: Three-dimensional sinogram files were created by binning the data into a total of 33 frames (six 30-sec frames, seven 60-sec frames, five 120-sec frames, four 300-sec frames, nine 600-sec frames, one 1200-sec frame and one 1800-sec frame). We applied a previously validated algorithm to list-mode data from the transmission scan to generate attenuation maps (Vandervoort and Sossi, 2008). This algorithm uses an analytical scatter correction, based upon the Klein-Nishina formula, for singles-mode transmission data. Following construction of the attenuation maps, emission list-mode

files were reconstructed using Fourier rebinning and filtered back projection, and corrected for normalization, dead time, scatter and attenuation, using software provided by Concorde Instruments (microPET Manager version 2.4.1.1). The resultant images had voxel dimensions of 0.949 mm x 0.949 mm x 1.212 mm and matrix dimensions of 128 x 128 x 63.

MRI Acquisition: Structural magnetic resonance (MR) images were acquired to allow for anatomically based demarcation of regions of interest (ROI). MR images were acquired one week after the PET scans. The monkeys received an intramuscular injection of ketamine hydrochloride (10 mg/kg) and atropine sulfate (0.01 mg/kg). Once the monkey was immobilized, an endotracheal tube was inserted to provide inhalation of 2-3% isoflurane gas (in 100% O₂) for the remainder of the scan. Each monkey was positioned on the bed of a 1.5 T Siemens scanner, with his head in the gantry, surrounded by an 8-channel, high-resolution, knee-array coil (Invivo Corporation). Nine T1-weighted volumes with three-dimensional, magnetization-prepared, rapid-acquisition, gradient-echo (MPRAGE) images were acquired (TR=1900 ms TE=4.38 ms, FOV=96 mm, flip angle 15 degrees, voxel size 0.5 mm, 248 slices, slice thickness 0.5 mm). Individual images were aligned to each other using Statistical Parametric Mapping 5 (Institute of Neurology, University College London, London, England), averaged together and resliced according to a previously developed MR template (Fears et al., 2009).

Data Processing: ROIs were drawn twice on each subject's structural MR image by a single experimenter blind to the subject identity using FSL View (FMRIB's Software Library v4.0). ROIs, sampled this way as replicates, included the whole caudate nucleus, putamen, ventral striatum and cerebellum.

1) ROI-based determination of binding potential (BP): Reconstructed PET images were corrected for motion and coregistered to the subject's MR image using the PFUS module within PMOD (version 3.15; PMOD Technologies). Using the ROIs, activity was extracted from the coregistered PET images and imported into the PMOD kinetic analysis program (PKIN). Time-activity curves were fit using the

Simple Reference Tissue Model (SRTM) (Lammertsma et al., 1996) to provide an estimate of k_2' , the rate constant of tracer transfer from the reference region to plasma. The k_2' estimates of the high-activity areas, i.e., the caudate nucleus and putamen, were averaged, and the time-activity curves refit using the SRTM2 model using the average, fixed k_2' value applied to all brain regions (Wu and Carson, 2002). BP was then calculated by subtracting 1.0 from the product of tracer delivery (R_1) and tracer washout (k_2'/k_{2a}). BPs from the left and right brain structures were averaged to create a single BP measurement for the caudate nucleus, putamen and ventral striatum. As the BP values of the ROI replicates were highly correlated, the BPs of the ROI replicates were averaged to obtain the final ROI-based measurements of D₂-like receptor availability in each of the brain regions.

- 2) Generation of Whole Brain BP Maps: Parametric binding maps, showing BP, were generated for each subject in PXM0D (PM0D), using the SRTM2 model with the same fixed k_2' values used above. This modeling requires time-activity data for low- and high-activity regions to generate the initial parameters for modeling. We used the activity in the putamen and cerebellum ROIs as the high- and low-activity references, respectively. In order to perform voxel-wise statistical analyses with BP maps, we realigned all BP maps to a study-specific MR template, which was created by sequentially registering each subject's skull-stripped MR scan (Multitracer, AIR version 5.0) using affine registration (FLIRT, FMRIB's Software Library v4.0), and creating an average of the registered images. Individual skull-stripped MR scans were then registered to the study-specific template space using affine registration (FLIRT). The resultant transformation matrix was applied to each individual subject's parametric binding map, which was previously registered to the individual subject's MRI. No additional smoothing was applied to the images.

Statistical Analyses: All statistical analyses were conducted using SPSS 15.0. Reliability of performance was examined by calculating Cronbach's alpha, a coefficient of reliability, for the number of trials

required to reach criterion in the acquisition, retention and reversal phases of the task during the first ten completed sessions. Paired-samples t-tests were conducted to examine the number of trials required to reach criterion in the acquisition and reversal phases, as well as the error types (neutral or perseverative) in the reversal phase of the task. Linear regressions were conducted to examine the relationships between D₂-like receptor availability and our behavioral measures; though we found significant linear relationships ($Y = a - bX$), visual inspection suggested that for some relationships an inverse function ($Y = a - b/X$) was more appropriate for the data. The asymptote (a) and slope (b) of each curve were estimated using the curve-fitting tool in SPSS. Models were compared using the Akaike Information Criterion (AIC) to determine whether the linear or inverse function best fit the data. When the inverse function was identified as the AIC-preferred model, the independent variables were transformed accordingly and correlations performed with the transformed values to calculate the Pearson correlation coefficient and significance values.

To examine the anatomical distribution of the relationship between positive-feedback sensitivity and BP within the striatum, linear regressions were performed using the FSL RANDOMISE v2.1 tool (Permutation-based nonparametric inference, Oxford University, Oxford UK) with a variance smoothing of 5 mm (FWHM Gaussian). A binary, striatal mask was created and feedback-sensitivity measures transformed according to the model that best fit the data according to our initial ROI analysis (see above). Threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009) was used to detect significant clusters of activation; this method provides the ability to perform cluster-based inference without the need to specify an arbitrary cluster-forming threshold, as is necessary when using Gaussian random field theory. For each analysis, 10,000 randomization runs were performed. Statistical maps were thresholded at $p < 0.05$ (two-tailed) and corrected for the search volume contained in the striatal mask.

Results

Behavioral Performance: Discrimination performance across the first ten acquisition, retention and reversal sessions completed by each subject showed a high degree of internal consistency, as indicated by the reliability coefficient, Cronbach's alpha, for acquisition (0.70), retention (0.76), and reversal performance (0.77). During the acquisition phase, the number of trials to reach criterion was 14.81 +/- 1.43 trials (mean +/- SE), which was significantly lower than the 25.69 +/- 3.86 trials (mean +/- SE) required to reach criterion during the reversal phase ($t(11)=-3.508$; $p<0.01$). Descriptive statistics for error type in the reversal phase indicated that the probability of making a response to the initially reinforced stimulus was significantly greater than that of making a response to the never rewarded stimulus ($t(11)=5.551$; $p < 0.001$). These results indicate that, although monkeys had been trained on multiple reversals, they still found the reversal phase of the task significantly more difficult than the acquisition of a novel stimulus-reward association. Performance during the first and the last completed reversal session were correlated ($r_{10}=0.613$; $p=0.03$), indicating that despite the multiple reversal sessions monkeys performed, the ability to flexibly modify behavior was reasonably trait-like.

D₂/D₃ Receptor Availability and Reversal-Learning Performance: Because technical delays resulted in subjects completing a different numbers of discrimination problems, we examined whether differences in the total number of discrimination problems completed by each subject was associated with either differences in D₂-like receptor availability in the striatal regions of interest, or average behavioral performance during the last three discrimination sets; no significant relationships were detected (all correlation |t|'s < 0.91). We also found no significant relationships between striatal D₂-like receptor availability and variation in the number of days between completion of the last discrimination problem and when PET scans were acquired (all correlation |t|'s < 2.09).

We then examined the relationship between D₂-like receptor availability in each of the three striatal regions and the average number of trials required to reach criterion for the last three

acquisition, retention and reversal sessions completed prior to PET scans. Because D₂-like receptor availability is negatively correlated with age in humans (Wang et al., 1995; Volkow et al., 1996b), we initially included age in the model as a covariate; however, because it was not a significant predictor in our dataset (possibly because the variation in age was restricted), it was removed from the model(s) and all other analyses.

As hypothesized, no significant relationship was found between the average number of trials required to reach criterion in the acquisition or retention phases and D₂-like receptor availability in any brain region assessed (all |t|'s < 1.29; Figures 1A and 1B). However, a relationship was found between the average number of trials required to reach criterion in the reversal session and receptor availability in the caudate nucleus ($r_{10}=-0.71$; $p=0.01$) and the putamen ($r_{10}=-0.67$; $p=0.02$), but not the ventral striatum ($r_{10}=0.28$; $p=0.38$) (Figure 1C). Specifically, greater D₂-like receptor availability in the caudate nucleus and putamen was associated with better reversal-learning performance, and this relationship was best modeled using an inverse function, as presented in Figure 1C (a solid line for the caudate nucleus and a dashed line for the putamen).

For the reversal phase, we examined whether D₂-like receptor availability in the caudate nucleus and putamen was correlated with specific response types normalized to the number of trials required to reach criterion. No significant relationship was found between D₂-like receptor availability in the caudate nucleus and the probability of making a correct response ($r_{10}=0.48$; $p=0.12$), a perseverative response ($r_{10}=-0.31$; $p=0.32$) or a neutral response ($r_{10}=-0.46$; $p=0.13$). Similarly, no significant relationships were found with D₂-like receptor availability in the putamen and the probability of making a correct response ($r_{10}=0.36$; $p=0.26$), a perseverative response ($r_{10}=-0.20$; $p=0.54$) or a neutral response ($r_{10}=-0.39$; $p=0.21$) (data not shown).

To ensure that the relationship was not specific to the last three discrimination problems completed, we examined the relationship between D₂-like receptor availability and the average number of trials required to reach criterion for all reversals completed for each subject. This assessment indicated that the average number of trials required to reach criterion across all the reversal sessions was correlated with D₂-like receptor availability in the caudate nucleus ($r_{10}=-0.68$; $p=0.01$) and putamen ($r_{10}=-0.56$; $p=0.05$) and the relationship was best described with an inverse function. It was present even in an examination of performance just on the first reversal completed ($r_{10}=-0.756$; $p=0.004$ for the caudate nucleus and $r_{10}=-0.778$; $p=0.003$ for the putamen).

D₂/D₃ Receptor Availability and Feedback Sensitivity in the Reversal Phase: We next examined the relationship between D₂-like receptor availability in the caudate nucleus, putamen and ventral striatum and the measures of behavioral sensitivity to feedback. The probability of following positive feedback with a correct response was correlated with D₂-like receptor availability in the caudate nucleus ($r_{10}=0.74$; $p=0.006$) and putamen ($r_{10}=0.74$; $p=0.006$) but not in the ventral striatum ($r_{10}=0.37$; $p=0.24$) (Figure 2A) (see statistical map, Figure 3). Correspondingly, the probability of following positive feedback with a perseverative response (regressive responding to the initially trained stimulus) was related to D₂-like receptor availability in the caudate nucleus ($r_{10}=-0.61$; $p=0.04$), but not in the putamen ($r_{10}=-0.47$; $p=0.12$) (Figure 2B). These relationships were best modeled with the inverse function as presented in Figure 2A and 2B: solid and dashed curves represent the relationship between feedback sensitivity and D₂-like receptor availability in the caudate nucleus and putamen, respectively. No significant correlations were found between D₂-like receptor availability and the probability of following positive feedback with a response to the never-rewarded stimulus. D₂-like receptor availability in the three striatal regions was not correlated with the probability of subjects following negative feedback with either the same incorrect response or a response to one of the two other stimuli (all correlation $|t|$'s < 0.45) (see Figure 2C).

Maps of the Relationship between Reversal-Learning Performance and D₂-like Receptor Availability:

Voxel-wise comparison revealed a significant negative correlation between the number of trials required to reach criterion in the reversal phase and D₂-like receptor availability, that extended throughout the caudate nucleus and putamen (Figure 3A). A similar negative correlation was found between the probability of following positive feedback with a perseverative response and D₂-like receptor availability in the dorsal striatum (Figure 3B). A moderate correlation was found between D₂-like receptor availability and the probability of following positive feedback with a correct response ($r_{10}=0.47$; $p=0.12$), but did not survive the TFCE-corrected $p < 0.05$ threshold. Significant statistical maps were overlaid to visualize the anatomical distribution of the significant relationships in the coronal (Figure 3C) and the transverse section (Figure 3D).

Exploratory analyses: Although D₂-like receptor availability was not correlated with the number of trials required to reach criterion during the acquisition of novel stimulus-reward associations, the strong correlation found with positive feedback-sensitivity measures warranted examination of the possible relationship of this receptor system with feedback sensitivity during acquisition. D₂-like receptor availability in the caudate nucleus, but not the putamen or ventral striatum, was linearly related to our measure of positive feedback sensitivity ($r_{10}=0.574$; $p=0.05$) (Figure 4) but was not with negative feedback sensitivity ($r_{10}=0.182$; $p=0.572$) (data not shown).

Discussion

This study demonstrated that D₂-like receptor availability within the dorsal aspects of the striatum was related to the ability to modify behavior during reversal learning, and to behavioral sensitivity to positive feedback. These results directly support the idea that the D₂-like receptor system is involved in the ability to shift responding when the association between a stimulus and reward is changed, and suggest that variation in reversal-learning performance reflects individual differences in sensitivity to positive feedback. These relationships are maintained under the conditions of natural variation, rather than manipulation, and together with studies in humans and rodents (Frank and O'Reilly, 2006; Boulougouris et al., 2009; Cools et al., 2009), provide powerful convergent evidence that the D₂-dependent dopamine signaling system is crucially involved in aspects of behavioral flexibility and reinforcement sensitivity.

D₂-Like Receptors and Reversal Learning: Experimental perturbations of D₂-like receptor signaling alter performance in tasks that require flexible modifications in behavior; these relationships hold in several species (Lee et al., 2007; Boulougouris et al., 2009; Cools et al., 2009; Herold, 2010) indicating that this receptor system represents a phylogenetically conserved mechanism for the rapid adjustment of behaviors. The findings presented here add an important dimension to prior experimental results by demonstrating that individual differences in the ability to update behavior in a reversal-learning task are related to natural variation in D₂-like receptor availability.

Our results provide evidence that the relationship between D₂-like receptor availability and reversal-learning performance is anatomically confined to the dorsal striatum, with no relationship found in the ventral striatum. These results are supported by data showing that a lesion of the dorsal, but not ventral, striatum impairs reversal learning in rats (Castane et al., 2010) and monkeys (Clarke et al., 2008). Moreover, activation of the dorsal striatum is observed in human subjects, studied with functional MRI, during a discrimination reversal task (Ghahremani et al., 2010). Though striatal

mechanisms may themselves be involved in reversal learning, there is also evidence that striatal D₂-like receptor availability is positively correlated with glucose metabolism in the orbitofrontal cortex (Volkow et al., 2000). Therefore, it is possible that striatal D₂-like receptor availability may be related to molecular and/or functional integrity of the orbitofrontal cortex, which in turn contributes to the correlations reported here.

The radioligand used in this study ([¹⁸F]fallypride) has equal affinity for both D₂ and D₃ receptor subtypes (Mukherjee et al., 1999), precluding assignment of the contributions of specific dopamine receptor subtypes. However, the relationships reported here were restricted to the dorsal striatum, an area with modest D₃ receptor expression relative to the ventral striatum (Bouthenet et al., 1991). Moreover, mice lacking the D₃ receptor exhibit enhanced reversal-learning performance (Glickstein et al., 2005) and administration of a D₃ agonist impairs reversal-learning performance in monkeys (Smith et al., 1999), suggesting that low D₃ receptor density would be expected to relate to reversal-learning performance in a manner opposite to that observed here. Therefore, the relationships reported in the current study are most likely due to variation in the D₂ receptor subtype. However, further studies using subtype-specific antagonists may help to clarify the validity of these hypotheses.

Taken with a host of pharmacological evidence from humans and rats (Boulougouris et al., 2009; Cools et al., 2009), these data suggest that individual differences in reversal-learning performance are a result of underlying variation in D₂-like receptor availability within the dorsal striatum. However, we cannot totally exclude the possibility that training history affected D₂-like receptor availability. It is also possible that variation in receptor availability detected in the current study is due to differences in endogenous dopamine levels acting in competition with the radioligand for the D₂-like receptor binding site, thereby influencing receptor availability measurements. Although we cannot reject this possibility, we believe it cannot fully account for the current findings. Based on evidence that striatal dopamine

synthesis is positively correlated with reversal-learning performance (Cools et al., 2009), a dominant influence of dopaminergic tone on D₂-like receptor availability would lead to a positive relationship with the number of trials required to reach criterion, opposite to our current findings. Therefore, we believe that the relationships presented in the current study are most likely due to variation in receptor level, and not to variation in dopamine levels; however, future studies examining D₂-like receptor availability in the absence of synaptic dopamine levels are needed to verify this hypothesis.

D₂-Like Receptors and Feedback Sensitivity: The ability to learn or reverse a stimulus-response association requires an integration of both positive and negative feedback in order to refine subsequent choices. Several lines of evidence support a crucial role for the dopamine system in these abilities. Schultz (1997) demonstrated that over the course of learning a stimulus-reward association, phasic firing of midbrain dopamine cells shifts from the time of reward presentation to the time of conditioned stimulus presentation. Subsequently, when a predicted reward is omitted, dopamine neuron activity declines below baseline (Hollerman and Schultz, 1998). Frank et al. (Frank et al., 2004) have argued that dopamine, acting on specific receptor subtypes that exhibit a segregated distribution on striatal medium spiny neurons, exerts dissociable actions in response to positive and negative feedback during learning. This theory posits that phasic release of dopamine, acting on medium spiny neurons in the direct pathway that express D₁ receptors, promotes learning from positive feedback, while declines in dopamine activity, locked to negative feedback, are hypothesized to release the D₂-expressing medium spiny neurons in the indirect pathway from inhibition via D₂ receptor signaling.

Here, however, we provide evidence that D₂-like receptor availability within the dorsal striatum is correlated with the ability of subjects to integrate positive, rather than negative, feedback in their ongoing choice behavior, which is surprising in light of the previously described theory. Notably, a recently developed neurocomputational model by Dreyer et al. (2010) suggested that both increases

and decreases in dopamine-neuron activity affect D₁- and D₂-like receptor function, albeit possibly to different degrees. Therefore, it is possible that the association between D₂-like receptor availability and positive feedback sensitivity stems from phasic dopamine release activating D₂ receptors as well as D₁ receptors. Dopamine acting on D₂-expressing medium spiny neurons may produce long-term depression in corticostriatal synapses on those neurons (Kreitzer and Malenka, 2007), reducing the strength of the indirect pathway that constrains behavior, thereby increasing the probability of making the same response on the following trial. Our results are consistent with deficits in positive feedback that have been reported in carriers of the A1 allele of the TaqIA polymorphism (Althaus et al., 2009; Jocham et al., 2009).

Here we report that D₂-like receptor availability is correlated with positive feedback sensitivity not only during the reversal of a stimulus-reward association, but also during its initial acquisition. Although the strength of the correlation is greatest in the reversal stage of the task, where strong expectancy violations may magnify underlying deficits in behavioral sensitivity to feedback, D₂-like receptors represent a principal substrate for explaining variation in positive feedback.

Implications for Addictive Disorders: Relatively low D₂-like receptor levels have been reported in several neuropsychiatric disorders, most prominently in substance abuse and dependence. Substance dependent individuals have lower D₂-like receptor availability (Volkow et al., 1993; Volkow et al., 1996a; Volkow et al., 2001b; Lee et al., 2009) and exhibit reversal-learning deficits (Fillmore and Rush, 2006; Salo et al., 2009; Ghahremani et al., 2011). Although animal studies have shown that chronic exposure to drugs can produce reductions in the striatal D₂-like receptor availability (Nader et al., 2006) and deficits in reversal learning (Jentsch et al., 2002), there is also evidence that lower D₂-like receptor levels may confer risk for behavioral dis-inhibition (Dalley et al., 2007) and drug self-administration (Nader et al., 2006; Dalley et al., 2007). Further, D₂-like receptor availability is correlated with known risk factors

for substance dependence, such as impulsivity (Lee et al., 2009; Buckholtz et al., 2010a) and novelty seeking (Zald et al., 2008; Huang et al., 2010), which are themselves associated with cognitive deficits (Cools et al., 2007; James et al., 2007).

We therefore propose that reversal-learning deficits, which measure behavioral inflexibility, represent an intermediary process between D₂-mediated transmission and behavior addictions. Further, pharmacological manipulations that increase D₂-mediated dopaminergic transmission, and improve behavioral flexibility, represent a principal treatment strategy for substance dependence, as behavioral flexibility is a known correlate of retention in a treatment program (Moeller et al., 2001; Aharonovich et al., 2006). Improving D₂-like receptor transmission also constitutes a plausible intervention strategy for individuals who are at high-risk for substance dependence and have cognitive impairments (Giancola et al., 1996a) that are predictive of greater substance use (Aytaclar et al., 1999).

Conclusion: Variation in D₂-like receptor availability in the dorsal striatum explains individual differences in behavioral flexibility and positive feedback sensitivity. Genetic influences that modulate D₂-like receptor expression and function in the dorsal striatum are therefore expected to influence impulsivity-like phenotypes and ultimately syndromes that involve impulse-control disorders. In this sense, D₂-dependent dopamine transmission may represent a final, common, biochemical pathway to manifestations of behavioral inflexibility across diagnostic categories.

Figures

Figure 2.1: The relationship between D₂-like receptor availability in the striatum (caudate nucleus, black circles; putamen, gray circles; and ventral striatum, open circles) and the average number of trials required to reach criterion during the acquisition phase (A), the retention phase (B), and the reversal phase (C). The inverse function best modeled the relationship between reversal learning and D₂-like receptor availability in the caudate nucleus (solid curve) and the putamen (dashed curve).

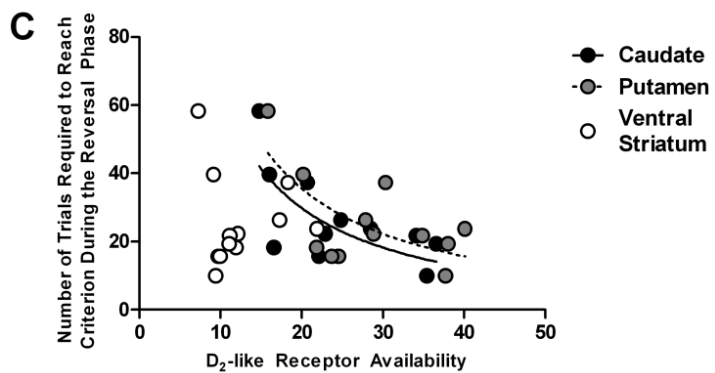
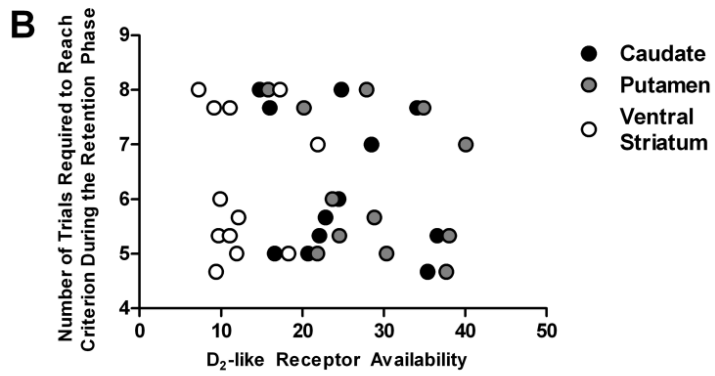
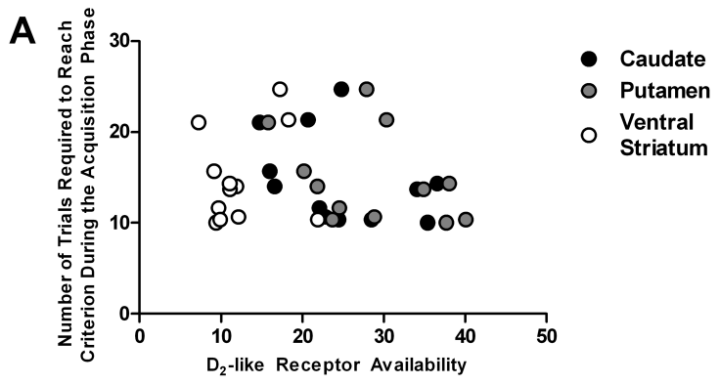


Figure 2.2: The relationship between D₂-like receptor in the three striatal regions (caudate nucleus, black circles; putamen, gray circles; and ventral striatum, open circles) and feedback sensitivity during the reversal phase. The probability of making a correct response following positive feedback was related to D₂-like receptor availability in the caudate nucleus (solid curve) and the putamen (dashed curve) (**A**), while the probability of making a perseverative response following positive feedback was related to D₂-like receptor availability in the caudate nucleus (solid curve) (**B**). No relationship was detected between the probability of making an incorrect response following negative feedback and D₂-like receptor availability in any of the striatal regions examined (**C**).

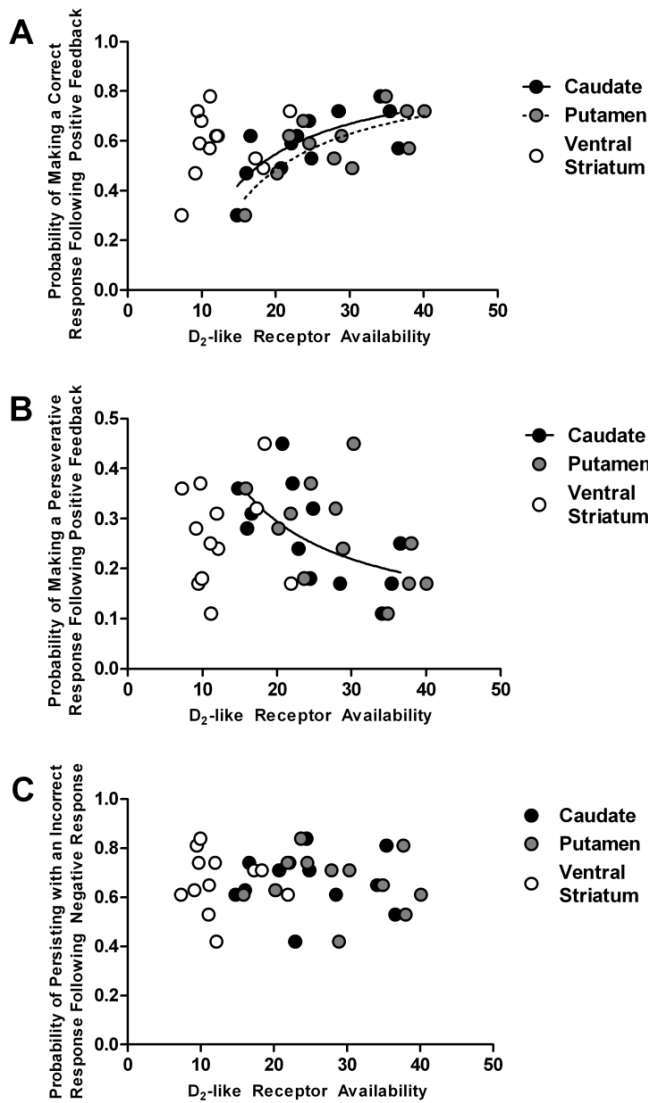


Figure 2.3: Statistical maps (p values) from the voxelwise regression of D₂-like receptor binding potential on the reversal-learning measures. The relationship between D₂-like receptor availability and the average number of trials required to reach criterion in the last three reversal sessions is presented in blue in **A**. The relationship between D₂-like receptor availability and the probability of following positive feedback with a perseverative response in the last three reversal sessions is presented in red in **B**. The overlap of the two statistical maps is presented in **C** (coronal plane) and **D** (transverse plane). TFCE images were overlaid on the study-specific MR template with results thresholded at TFCE-corrected $p < 0.05$.

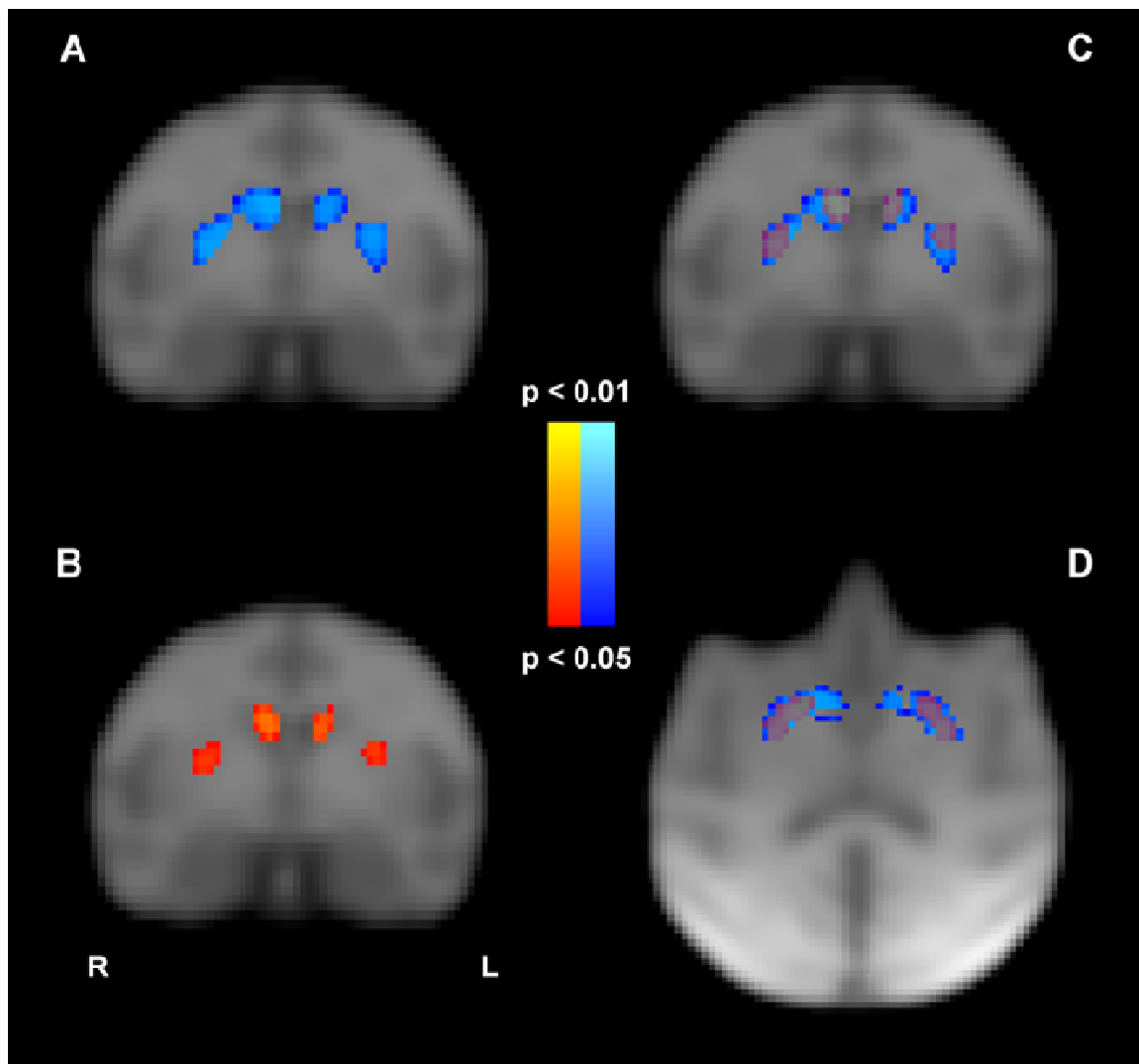
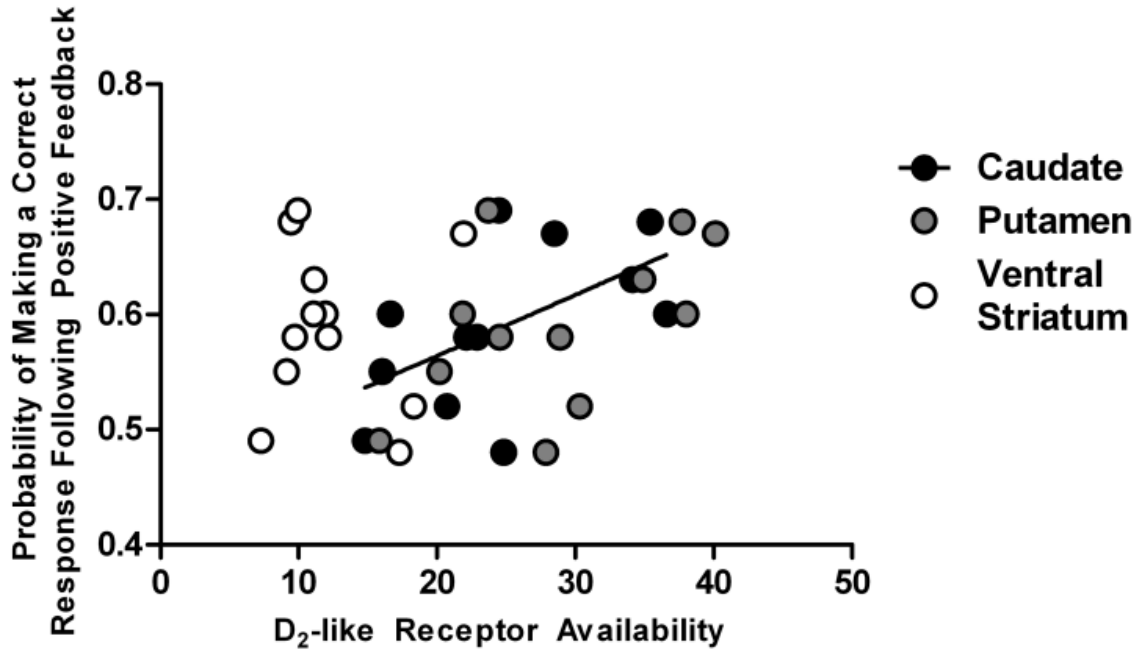


Figure 2.4: The relationship between D₂-like receptor availability in the caudate nucleus (black circles), putamen (gray circles), or ventral striatum (open circles) and the probability of making a correct response following positive feedback during the acquisition of stimulus–reward association is shown. The linear relationship between the probability of making a correct response following positive feedback and D₂-like receptor availability in the caudate nucleus is represented by the black line.



Tables:

Table 2.1: The total number of discrimination problems, the number of days between last completed discrimination session and assessment of D₂-like receptor availability, and the average number of trials required to reach criterion in the last three reversal phases for each subject.

Table 1: The total number of discrimination problems, the number days between last completed discrimination session and assessment of D₂-like receptor availability, and the average number of trials required to reach criterion in the last three reversal phases for each subject.

Subject	A9727	C9726	D9802	E0241	G0261	H0271	I0221	J0247	K0488	L0441	M0457	N0406
Total number of completed discrimination sessions	21	20	21	14	10	14	14	14	17	17	17	17
Days between last completed discrimination session and PET scan	14	7	21	36	14	4	42	3	33	32	40	39
Average number of trials required to reach criterion in the last three reversal phases	39.67	26.33	58.33	23.67	15.67	22.33	37.33	18.33	10	21.67	19.33	15.67

Chapter 3

Dysregulation of D₂-Mediated Dopamine Transmission in Monkeys after Chronic Escalating Methamphetamine Exposure

Summary

Compulsive drug-seeking and drug-taking are important substance-abuse behaviors that have been linked to alterations in dopaminergic neurotransmission and to impaired inhibitory control. Evidence supports the notions that abnormal D₂ receptor-mediated dopamine transmission and inhibitory control may be heritable risk factors for addictions, and that they also reflect drug-induced neuroadaptations. To provide a mechanistic explanation for the drug-induced emergence of inhibitory-control deficits, this study examined how a chronic, escalating-dose regimen of methamphetamine administration affected dopaminergic neurochemistry and cognition in monkeys. Dopamine D₂-like receptor and dopamine transporter (DAT) availability and reversal-learning performance were measured before and after exposure to methamphetamine (or saline), and brain dopamine levels were assayed at the conclusion of the study. Exposure to methamphetamine reduced dopamine D₂-like receptor and DAT availability, and produced transient, selective impairments in the reversal of a stimulus-outcome association. Furthermore, individual differences in the change in D₂-like receptor availability in the striatum were related to the change in response to positive feedback. These data provide evidence that chronic, escalating-dose methamphetamine administration alters the dopamine system in a manner similar to that observed in methamphetamine-dependent humans. They also implicate alterations in positive-feedback sensitivity associated with D₂-like receptor dysfunction as the mechanism by which inhibitory control deficits emerge in stimulant-dependent individuals. Finally, a significant degree of neurochemical and behavioral variation in response to methamphetamine was detected, indicating that individual differences affect the degree to which drugs of abuse alter these processes. Identification of

these factors ultimately may assist in the development of individualized treatments for substance dependence.

Introduction

Difficulties with exerting inhibitory control over pre-potent behaviors has been proposed as a central feature of substance dependence (Jentsch and Taylor, 1999). Support for this hypothesis comes from observations that individuals dependent upon illicit substances exhibit inhibitory control deficits (Fortier et al., 2008; Ersche et al., 2011; Ghahremani et al., 2011), suggesting that these impairments represent a dimension of behavioral dysfunction common to many forms of addiction. Although pre-existing inhibitory control deficits may be a risk factor for the development of substance dependence (Dalley et al., 2007; Romer et al., 2009), there is evidence that these deficits can result from drug exposure (Jentsch et al., 2002; Stalnaker et al., 2009; Izquierdo et al., 2010; Porter et al., 2011). However, the neural adaptations consequent to drug use that elicit inhibitory control deficits remains an open research question.

Dysregulation of the dopamine D₂-like (D₂ and D₃) receptor system has emerged as a candidate mechanism underlying difficulties with inhibitory control in addictions (Groman and Jentsch, 2011a; Izquierdo and Jentsch, 2011). Stimulant-dependent individuals exhibit abnormally low striatal D₂-like receptor availability (Volkow et al., 1993; Volkow et al., 2001b; Lee et al., 2009), and these deficits may result from drug exposure itself (Nader et al., 2006). Outside the context of experience with drugs, pharmacological blockade of D₂-like receptors results in inhibitory control deficits (Lee et al., 2007; Herold, 2010), and variation in D₂-like receptor availability is correlated with individual differences in measures of inhibitory control (Groman et al., 2011). Therefore, dysfunction of D₂-like receptor transmission may underlie the deficits in inhibitory control that emerge in drug-dependent individuals.

Some of the alterations in dopaminergic markers observed in methamphetamine-dependent individuals (McCann et al., 1998; Lee et al., 2009) can be modeled with high-dose methamphetamine administration (Wagner et al., 1980; Villemagne et al., 1998). However, much of the long-term impact of realistic patterns of slow, escalating-dose exposure to methamphetamine, which produces tolerance

that protects against drug-induced neurotoxicity (Segal et al., 2003), on the integrity of the dopamine system remains unknown. Additionally, the characteristics of cognitive dysfunction in methamphetamine-dependent persons are not consistent (Price et al., 2011), possibly because of variability in drug histories and individual differences. Finally, measures of inhibitory control have not been combined with biochemical and neuroimaging assessments within the same subjects, limiting our understanding of the complex relationships between these measures.

To bridge these gaps, the current study used positron emission tomography (PET) to quantify D₂-like receptor and DAT availability in monkeys tested for their abilities to acquire, retain, and reverse visual discriminations before and after a 31-day escalating dose-regimen of methamphetamine (or saline). The *in vivo* phase of this experiment was followed by *ex vivo* measurements of dopamine and dopamine-related metabolites in brain regions involved in inhibitory control. Based on the available evidence, we hypothesized that chronic methamphetamine would reduce striatal D₂-like receptor and DAT availability and dopamine levels, and would produce behavioral deficits specific to the reversal phase of the discrimination task.

Methods

Subjects: Fourteen male vervet monkeys (*Chlorocebus aethiops sabaesus* from the UCLA Vervet Research Colony), ranging from 5 to 9 years of age, were included in this study. Monkeys were individually housed in a climate-controlled vivarium, where they had unlimited access to water and received twice-daily portions of standard monkey chow in amounts that exceeded their nutritional needs (Teklad, Madison, WI). All of the subjects were able to see, hear and communicate with other individuals in the room. After behavioral testing was conducted in the morning, the monkeys received half of their daily portion of allotted chow (at approximately 1100 h); their second half was provided in the afternoon (at approximately 1500 h). The total amount of chow received was never reduced during the experiment to increase motivation for task performance. Two monkeys were excluded from some aspects of the study for unrelated reasons: one exhibited anxiety that compromised task performance, and was only used for the imaging and biochemical assays and one did not receive a baseline PET scan for D₂-like receptor availability due to technical problems.

All monkeys were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Drugs: Methamphetamine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). Doses of methamphetamine were prepared fresh daily in sterile normal saline, and the solution was filtered through 22- μ m Millex syringe filters (Millipore). All injections were administered intramuscularly at a volume of 0.1 mL/kg.

Dosing regimen: The dosing regimen was designed to model the escalation in both frequency of intake and cumulative daily dose reported by human users of methamphetamine (Han et al., 2011). Table 1

provides details of the 31-day regimen used. The initial dose was administered at 0830 h. During week two, a second dose was administered at 1630 h. During weeks three through five, the second dose was administered at 1330 h, and a third was given at 1630 h. For the last two weeks of treatment, the second dose was administered at 1100 h, the third at 1330 h, and a fourth at 1600 h.

Discrimination Acquisition, Retention and Reversal Learning: Monkeys were first trained to move from their individual cages into a transport cart, and were brought to a quiet testing room where the transport cart was aligned with a Wisconsin General Testing Apparatus. The general procedures used in the current study have been described previously (Groman et al., 2011).

Monkeys were trained to acquire, retain and reverse novel visual discriminations. Testing sessions began when the experimenter raised an opaque screen, allowing the monkey access to three boxes, each fitted with a novel visual stimulus on top. During each trial, the monkey was allowed to open a single box. A trial ended after a correct choice of the rewarded visual stimulus (reward was a small piece of apple, orange, grape or raisin), an incorrect choice, or an omission (no response for 2 min), and a 20-s inter-trial interval followed. The spatial positions of the different visual stimuli were varied pseudorandomly across trials.

The first session of a discrimination problem was the acquisition phase, during which the monkey was presented with three novel stimuli, and had to learn which one was associated with reward on the basis of trial and error. Once a performance criterion was met (seven correct choices in ten consecutive trials), the session ended and the monkey was returned to its home cage. If the criterion was not met within 80 trials, the session ended and the same discrimination problem was presented the following day until the criterion was met. No monkeys required more than 2 days of acquisition training.

One day after reaching the acquisition criterion, monkeys were tested in a retention phase, where the stimulus-reward contingencies remained unchanged, and again the monkeys were required

to meet a performance criterion (four correct responses in five consecutive trials). Immediately after reaching this criterion, the reversal phase began, with no explicit signal of the transition given to the monkey. In the reversal phase, the stimulus that was rewarded previously was no longer rewarded while one of the previously non-rewarded stimuli became rewarded. Designation of the newly rewarded stimulus was counterbalanced across subjects. The session continued, only for that day, until the monkey met the performance criterion (seven correct choices out of ten consecutive trials) or 70 trials had been completed, whichever occurred first.

The number of trials required to reach criterion and number of correct responses in the acquisition, retention and reversal phases were the primary dependent measures. For the reversal phase, the number of responses directed at the previously rewarded stimulus (perseverative responses) and the number of responses directed at the never rewarded stimulus (neutral responses) were also recorded. The frequency of each type of response was also calculated by dividing the number of correct, perseverative or neutral responses by the total number of trials completed in the reversal phase.

Subjects were trained on several novel discrimination problems before beginning the treatment regimen. The baseline data for twelve of the fourteen monkeys used in the current study have been presented in detail elsewhere (Groman et al., 2011). Assignment of monkeys to the saline group (N=7) or the methamphetamine (N=7) group was balanced on the basis of average performance in the acquisition, retention and reversal phases of the last three visual discrimination problems completed immediately prior to beginning the dosing regimen.

Drug effects on behavioral performance were determined by comparing data at three time-points, each assessment carried out using novel stimuli and the same performance criteria: 1) performance derived from the discrimination problem completed immediately before the treatment regimen was started (referred to as the “baseline assessment”), 2) performance during 3 weeks within

the course of the treatment regimen (referred to as the “3-week assessment”); to limit the anorectic effects of methamphetamine, this test occurred after at least 36 h had elapsed since the last methamphetamine (or saline) administration, and 3) performance at 5 days after the last drug administration (referred to as the “five-days post-exposure assessment”).

After completion of the dose regimen, two additional behavioral assessments were conducted, but the performance criterion was increased (nine correct choices in ten completed trials for the acquisition, retention and reversal phases) to augment the cognitive demands of the task. Specifically, the additional acquisition and retention training was expected to increase the likelihood of perseverative responding; these two “high-difficulty” sessions were conducted at 8 days and 2 weeks after cessation of drug administration (referred to as the “8-day post-exposure assessment” and the “2-week post-exposure assessment”, respectively).

MRI Scanning Procedures: One week after the acquisition of baseline PET scans, structural magnetic resonance (MR) images were acquired on a 1.5 T Siemens scanner to allow for anatomically based demarcation of regions of interest using procedures identical to those previously described (Groman et al., 2011). A second MR image was acquired approximately 3½ weeks after completion of the dosing regimen; however, only the first MR image was used in the current study for co-registration purposes.

PET Scanning Procedures: PET scanning was performed using [¹⁸F]fallypride and [¹¹C]WIN-35,428 as radioligands for measurements of dopamine D₂-like receptors and DAT availability, respectively. Three PET scanning sessions were conducted: 1) approximately 2 weeks before initiating drug administration (referred to as the “baseline scan”), 2) approximately 2 weeks after cessation of drug administration, to examine the immediate effects of methamphetamine (referred to as the “2-week post-exposure scan”) and 3) 7 weeks after cessation of drug administration to examine the stability of neural changes and the potential for recovery (referred to as the “7-week post-exposure scan”). All scans were conducted using

a MicroPET Model P4 scanner (Concorde Instruments, Knoxville, TN), with procedures previously described (Groman et al., 2011).

Monkeys received an intramuscular injection of ketamine hydrochloride (10 mg/kg) and glycopyrrolate (0.01 mg/kg). Once monkeys were sedated, an endotracheal tube was placed to provide inhalation of 2-3% isoflurane (in 100% O₂). Vital signs (heart rate, respiratory rate, oxygen saturation and temperature) were monitored and recorded every 15 min throughout the scan. A tail-vein catheter was placed, and the monkey was positioned on the scanning bed such that the imaging planes were parallel to the orbitomeatal line and the top of the head was at the front of the field of view. A 20-minute ⁶⁸Ge transmission scan was acquired before administration of the radioligand for attenuation correction. All subjects received a bolus injection [¹¹C]WIN 35428 (1.0 mCi/kg), followed by a 5-mL saline flush, and data were acquired for 90 min. When radioactivity had fallen to baseline levels (~3 h after [¹¹C]WIN-35,428 administration), a bolus injection of [¹⁸F]fallypride was delivered (0.3 mCi/kg), followed by a 5-mL saline flush. Dynamic data were acquired in list mode for 180 min. After the scan, gas anesthesia was removed and the animals were allowed to recover overnight before being returned to their home cages.

Quantification of dopamine and dopamine metabolites: Approximately 2-6 months after completing the dose regimen, monkeys were chemically restrained with ketamine hydrochloride (10 mg/kg, i.m.) and heavily sedated with sodium pentobarbital (30 mg/kg, i.v.). Following loss of the corneal reflex, they were transcardially perfused with ice-cold saline for 10 min; their brains were then removed and regionally dissected on a cold platform. The tissue was immediately frozen on dry ice and stored at -80^o C until assayed. Tissue was processed by high-pressure liquid chromatography, as previously described (Jentsch et al., 1997).

Based on previous studies, we hypothesized that dopamine and dopamine-related metabolites (HVA – homovanilic acid; DOPAC – 3,4-dihydroxyphenylacetic acid; 3-MT – 3-methoxytyramine) would

be most affected by exposure to methamphetamine within the striatum (Melega et al., 2008). Therefore, we restricted our analyses to dopamine turnover ratios (tissue content of each metabolite [in ng/mg protein]/tissue content of parent amine [ng/mg protein]) within the following brain regions: caudate nucleus, putamen, nucleus accumbens core and nucleus accumbens shell. In regions where dopamine turnover differed between methamphetamine- and saline-exposed monkeys, we conducted additional separate analyses on levels of dopamine and the related metabolite to further explore observed differences.

Data Processing

Feedback-Sensitivity Measures: Feedback sensitivity was determined by examining the choice behavior of subjects on a trial-by-trial basis during the acquisition and reversal phases of the discrimination sessions. First, trials were categorized on the basis of whether the subjects received positive or negative feedback on the preceding trial (i.e., rewarded or not). Following a positive-feedback trial, in which a correct response was made, the subsequent response was classified either as another correct response or an incorrect response. For the reversal-phase data, we further classified an incorrect response as a response directed at the previously rewarded stimulus, or a response to the stimulus that was never rewarded. Following negative feedback, when an incorrect response was made, the subsequent response was classified either as the same incorrect response or a response directed at one of the other two stimuli, irrespective of whether the response was correct or incorrect. The total number of these individual types of responses made after positive or negative feedback was determined, and their frequencies calculated by dividing these totals by the total number of positive or negative events, respectively, for the acquisition and reversal phase of each discrimination session.

Reconstruction of PET images: Three-dimensional sinogram files were created by binning the data into 18 frames (four 60-sec frames, three 120-sec frames, six 300-sec frames, and five 600-sec frames) for

[¹¹C]WIN-35,428 sinograms and 33 frames (six 30-sec frames, seven 60-sec frames, five 120-sec frames, four 300-sec frames, nine 600-sec frames, one 1200-sec frame and one 1800-sec frame) for [¹⁸F]fallypride sinograms. We applied a previously validated algorithm to the transmission scan list-mode data to generate attenuation maps (Vandervoort and Sossi, 2008). This algorithm uses an analytical scatter correction, based upon the Klein-Nishina formula, for singles-mode transmission data. Following construction of the attenuation maps, emission list-mode files were reconstructed using the Ordered-Subsets Expectation Maximization (OSEM) algorithm, and were corrected for normalization, dead time, scatter and attenuation using software provided by the scanner manufacturer (microPET Manager version 2.4.1.1). The resultant images had voxel dimensions of 0.949 mm x 0.949 mm x 1.212 mm and matrix dimensions of 128 x 128 x 63.

PET Data Processing: Using FSL View (FMRIB's Software Library v4.0), the following regions of interest (ROIs) were drawn on the structural MR image of each subject by a single experimenter blind to the subject's identity: the caudate nucleus, putamen, ventral striatum, ventral mesencephalon and cerebellum.

Reconstructed PET images were corrected for motion using the realignment function within Statistical Parametric Mapping 5 (Institute of Neurology, University College London, London, England). Each subject's MR image was then co-registered to the PET image using the Automated Image Registration program (AIR version 5; (Woods et al., 1993), and the resultant transformation was applied to the ROIs. ROIs in the native PET space were then used to extract activity from the PET images, and the activities were imported into the PMOD kinetic analysis program (PKIN). Time-activity curves were fit using the Multilinear Reference Tissue Model (MRTM) (Ichise et al., 2003) to provide an estimate of k_2' . The k_2' estimates of the high-activity areas, i.e., the caudate nucleus and putamen, were averaged, and time-activity curves were refit with MRTM2 using the average fixed k_2' value applied to all brain regions

and a fixed starting point of 27.5 minutes. Binding potentials from the left and right brain structures were averaged to create single measurements for each ROI.

Statistical Analyses: Initial inspection of the PET and behavioral measures revealed significant violations of sphericity and non-normality of data distributions, which violated the general assumptions of general linear models. These violations prevented the use of statistical models, such as a repeated-measures analysis of variance or an analysis of covariance for these data, which require sphericity and normal distribution of data. All analyses were conducted using generalized estimating equations (Avants et al.), a quasi-likelihood based, population-averaged model that allows for variance of the dependent variable to be a specified function of the mean (to account for non-normal distribution) and for correlated datasets and non-linear relationships between its mean and the set of linear predictors (Ballinger, 2004). Where applicable, longitudinal data (i.e., PET and behavioral data) were entered into the model as repeated measures, and experimental group was included as a between-subjects factor. For the PET data, measurements calculated for a given ROI in the 3 scans were entered as repeated measures, with experimental group as a between-subjects factor. The covariance structure was determined on the basis of goodness-of-fit estimates (Quasi Likelihood under Independence Model Criterion), and probability distribution and link functions chosen on the basis of known properties of the type of variable or through exploratory analysis of descriptive qualities of data distributions. PET data were analyzed using an unstructured working correlation matrix and an inverse Gaussian distribution with the identity link function. Behavioral data were analyzed using an unstructured working correlation matrix and a negative binomial distribution with a log link function. Finally, for the *ex vivo* data, dopamine turnover was analyzed using an unstructured working correlation matrix and normal distribution with the identity link function. Dopamine and HVA levels were analyzed using an unstructured correlation matrix and a gamma distribution with the identity link function. Significant interactions were explored using post hoc, pair-wise comparisons with a Bonferroni adjustment to correct for multiple comparisons. Because

saline-exposed monkeys exhibited changes in the behavioral and neurochemical measures over time, pair-wise comparisons were conducted considering the baseline measures for each subject, rather than employing simple between-groups contrasts at each time point. Spearman's rank correlation coefficient (Spearman's rho) was used to assess the statistical dependence of PET and behavioral measures. The number of days since completion of the treatment regimen was included as a covariate in the post-mortem analyses.

Results

D₂-like Receptor Availability: There were no significant group differences (saline vs. methamphetamine) prior to treatment in any of the brain regions examined (all p 's > 0.35). However, drug exposure significantly affected D₂-like receptor availability in the caudate nucleus (group: Wald χ^2 with 1 df = 0.030, p = 0.86; scan: Wald χ^2 with 2 df = 31.59, p < 0.001; group by scan: Wald χ^2 with 2 df = 48.91, p < 0.001), putamen (group: Wald χ^2 with 1 df = 0.001, p = 0.98; scan: Wald χ^2 with 2 df = 37.96, p < 0.001; group by scan: Wald χ^2 with 2 df = 54.94, p < 0.001) and ventral striatum (group: Wald χ^2 with 1 df = 0.012, p = 0.91; scan: Wald χ^2 with 2 df = 9.20, p = 0.01; group by scan: Wald χ^2 with 2 df = 24.95, p < 0.001), but not in the ventral mesencephalon (group: Wald χ^2 with 1 df = 0.58, p = 0.45; scan: Wald χ^2 with 2 df = 10.29, p = 0.006; group by scan: Wald χ^2 with 2 df = 1.91, p = 0.38). *Post-hoc* analyses confirmed that D₂-like receptor availability had decreased from baseline levels in only the caudate nucleus and putamen at the 2-week post-treatment scan in methamphetamine-exposed monkeys (all p 's < 0.001); however, no significant pair-wise comparisons were detected in the ventral striatum (all p 's > 0.11). The reductions in D₂-like receptor availability in both the caudate nucleus and putamen were still present at the 7-week post-exposure scan (all p 's < 0.001), despite a significant increase between the 2-week and 7-week post-exposure scans in both regions (all p 's < 0.001) (Figure 1A and 1B). The saline-exposed group exhibited an unexpected increase in D₂-like receptor availability in the caudate and putamen at the 2-week post-exposure scan (p < 0.001), an effect that remained at the 7-week post-exposure scan (p < 0.001); these effects could reflect the low-level stress associated with daily saline injections.

DAT Availability: Similar to findings on D₂-like receptor availability, no significant group differences (saline vs. methamphetamine) in DAT availability were detected before treatment in any of the brain regions examined (all p 's > 0.30). However, drug exposure altered DAT availability in the caudate nucleus (group: Wald χ^2 with 1 df = 4.54, p = 0.03; scan: Wald χ^2 with 2 df = 56.75, p < 0.001; group by scan: Wald χ^2 with 2 df = 20.67; p < 0.001), putamen (group: Wald χ^2 with 1 df = 8.16, p = 0.004; scan: Wald χ^2 with 2 df

=52.38, $p < 0.001$; group by scan: Wald χ^2 with 2 df =91.37, $p < 0.001$), ventral striatum (group: Wald χ^2 with 1 df =3.62, $p = 0.06$; scan: Wald χ^2 with 2 df =44.07, $p < 0.001$; group by scan: Wald χ^2 with 2 df =17.68, $p < 0.001$) and ventral mesencephalon (group: Wald χ^2 with 1 df =1.57, $p = 0.21$; scan: Wald χ^2 with 2 df =24.29, $p < 0.001$; group by scan: Wald χ^2 with 2 df =9.62, $p = 0.008$). Post-hoc analyses revealed that methamphetamine-exposed monkeys exhibited significant reductions in DAT availability at the 2-week post-exposure scan in the caudate nucleus, putamen and ventral striatum (all p 's < 0.001), but not in the ventral mesencephalon ($p = 0.10$). The reduction in DAT availability in methamphetamine-exposed monkeys, relative to baseline, persisted at the 7-week post-exposure time-point in the caudate nucleus and putamen (all p 's < 0.001) although there was a significant increase in DAT availability at the 7-week post-exposure scan relative to the 2-week post-exposure scan (all p 's < 0.001). Reductions in DAT availability in the ventral striatum of methamphetamine-exposed monkeys were still present at the 7-week post-exposure scan ($p < 0.001$), with no significant recovery between the 2-week and 7-week post-exposure scan ($p = 0.38$). An unexpected reduction in DAT availability, compared to baseline, at the 2-week post-exposure scan was found in saline-exposed monkeys in the ventral striatum ($p = 0.03$), but not in the caudate nucleus, putamen or ventral mesencephalon (all p 's > 0.90). However, no significant changes from baseline in DAT availability were detected in saline-exposed monkeys at the 7-week post-exposure scan in any of the brain regions (all p 's > 0.90).

Behavior: Group assignment on the basis of baseline performance achieved balance between the two groups: the average number of trials required to reach criterion in the acquisition, retention and reversal phase of the last three visual discrimination sessions was not significantly different between groups (all p 's > 0.30).

Analysis of behavior before, during and after drug administration revealed no significant differences between groups for the number of trials required to reach criterion in the acquisition (group:

Wald χ^2 with 1 df=1.38, p=0.24; discrimination session: Wald χ^2 with 2 df=2.70, p=0.26; group by discrimination session: Wald χ^2 with 2 df=0.14, p=0.93; Figure 3A) or in retention phases (group: Wald χ^2 with 1 df=0.35, p=0.56; discrimination session: Wald χ^2 with 2 df=0.80, p=0.67; group by discrimination session: Wald χ^2 with 2 df=0.17, p=0.92; Figure 3B). However, trials required to reach criterion in the reversal phase significantly diverged between groups across subsequent behavioral assessments points (group: Wald χ^2 with 1 df=0.00, p=0.99; discrimination session: Wald χ^2 with 2 df=13.79, p=0.001; group by discrimination session: Wald χ^2 with 2 df=67.56, p<0.001; Figure 3C). Post-hoc analyses confirmed that this was due to an increase in the number of trials required to reach criterion in methamphetamine-exposed monkeys between baseline and the 3-week assessment (p<0.001); however, no other pair-wise comparisons were significant (all p's>0.81), indicating that this behavioral effect was transient. Table 2 presents the number of trials required to reach criterion for each subject during the reversal phase of each discrimination session completed.

Relating changes in D₂-like receptor availability with behavior: We previously reported that individual differences in D₂-like receptor availability within the dorsal striatum were correlated with reversal-learning performance and sensitivity to positive but not negative feedback (Groman et al., 2011). Therefore, we expected that the change in D₂-like receptor availability within the dorsal striatum across scans would be correlated with the change in positive-feedback sensitivity, irrespective of drug exposure.

To test this hypothesis, the relationship between difference scores for D₂-like receptor availability in the dorsal striatum (2-weeks post-exposure scan - baseline) and feedback-sensitivity measures gathered before and after treatment during the reversal phases (5-days post-exposure assessment - baseline) were examined. Consistent with previous results, changes in D₂-like receptor availability (in any of the brain regions examined) were not correlated with the change in negative-

feedback sensitivity in the reversal phases (all $p's > 0.23$). However, the change in the frequency with which a monkey persisted with a correct response following positive feedback was positively correlated with the change in D₂-like receptor availability in the caudate nucleus (Spearman's $\rho = 0.587$, $p = 0.04$; Figure 4A). To examine whether this relationship was present in methamphetamine- and saline-exposed groups, the correlation coefficients for methamphetamine- and saline-exposure groups were compared using the Fisher transformation. The correlation coefficients did not differ statistically between the groups (methamphetamine-exposed monkeys: Spearman's $\rho = 0.571$; saline-exposed monkeys: Spearman's $\rho = 0.60$; $z = -0.06$, $p = 0.95$), indicating that the change in D₂-like receptor was positively correlated with change in behavior, regardless of group.

To examine the persistence of this relationship, the same analysis was conducted using difference scores, from baseline, for the 7-week post-exposure D₂-like receptor availability measurements and the last behavioral assessment conducted. The change in the frequency with which a monkey persisted with a correct response following positive feedback was positively correlated with the change in D₂-like receptor availability in the caudate nucleus (Spearman's $\rho = 0.60$; $p = 0.03$; Figure 4B), and the correlation coefficients did not differ between the groups (methamphetamine-exposed monkeys: Spearman's $\rho = 0.54$; saline-exposed monkeys: Spearman's $\rho = 0.54$).

Relating changes in DAT availability with behavior: To examine the specificity of the above-mentioned relationships, the same analyses were conducted with the change in DAT availability (2-week post-exposure scan – baseline) and the change in positive-feedback sensitivity in the reversal phases (5-day post-exposure assessment – baseline). The change in DAT availability within the putamen was negatively correlated with change in the frequency of monkeys choosing the never-rewarded stimulus following positive feedback (Spearman's $\rho = -0.65$; $p = 0.02$). No other significant correlations were detected (all $p's > 0.05$).

Quantification of dopamine and dopamine metabolites: Table 3 outlines the results from the *ex vivo* tissue analysis of the four striatal regions examined in this study. Dopamine turnover with levels of HVA and DOPAC, but not 3-MT, was higher in methamphetamine-exposed monkeys, compared to saline-exposed monkeys, in the caudate nucleus (HVA/dopamine: Wald χ^2 with 1 df=9.05, p=0.003; DOPAC/dopamine: Wald χ^2 with 1 df =3.16, p=0.08), putamen (HVA/dopamine: Wald χ^2 with 1 df =10.81, p=0.001; DOPAC/dopamine: Wald χ^2 with 1 df =6.42, p=0.01) and nucleus accumbens core (HVA/dopamine: Wald χ^2 with 1 df =9.51, p=0.002; DOPAC/dopamine: Wald χ^2 with 1 df =10.98, p=0.001). Further analyses indicated that these effects were the result of lowered dopamine levels in methamphetamine-exposed, compared to saline-exposed, monkeys within the putamen (mean +/- SEM saline: 110 +/- 17 ng/mg, methamphetamine: 59 +/- 4.16 ng/mg; Wald χ^2 with 1 df =9.75, p=0.002) and nucleus accumbens core (mean +/- SEM saline: 77 +/- 9.8 ng/mg, methamphetamine: 42 +/- 2.87 ng/mg; Wald χ^2 with 1 df =14.81, p<0.001), rather than differences in DOPAC or HVA levels (all p's>0.14). Dopamine or HVA levels in the caudate did not differ significantly between methamphetamine- and saline-exposed monkeys (all p's>0.39).

Discussion

The findings reported here provide direct evidence that exposure to an escalating dose regimen of methamphetamine administration produces changes to the dopamine system that mirror those that have been previously observed in human methamphetamine abusers (Volkow et al., 2001b; Lee et al., 2009). By combining a chronic, escalating dose regimen that models human methamphetamine abuse more closely than the acute, large-dose regimens previously used, with a longitudinal study design, we observed that a 31-day escalation of methamphetamine administration significantly reduces striatal D₂-like receptor, DAT availability and dopamine tone. The persistence of these effects following 7 weeks of abstinence indicates that though some recovery occurs, as in humans (Volkow et al., 2001a), it is limited and proceeds slowly. Furthermore, methamphetamine administration produced transient behavioral deficits that were linked to the observed changes in the dopaminergic system.

Escalation of methamphetamine administration results in diminished striatal D₂-like receptor availability,

DAT availability and dopamine levels: In line with the current results, previous evidence indicates that dysfunction of the dopamine system is a neurochemical consequence of methamphetamine exposure. High doses of methamphetamine rapidly reduce striatal DAT density/availability and dopamine tone in rodents and primates (Fleckenstein et al., 1997; Melega et al., 1998), paralleling the abnormalities found in humans who abuse methamphetamine. However, inconsistent results have been found with respect to D₂-like receptors. While research participants who abuse methamphetamine exhibit abnormally low D₂-like receptor availability, when compared to controls (Volkow et al., 2001b; Volkow et al., 2001a; Lee et al., 2009), there are discrepancies in the literature as to whether this effect is a consequence of methamphetamine (Schmidt et al., 1985; Melega et al., 2008). The data presented here suggest that methamphetamine-induced reductions in D₂-like receptors emerge during the course of extended exposure and that these reductions persist beyond the duration of acute, high-dose administration of the drug (McCabe et al., 1987).

The mechanism by which methamphetamine alters the dopamine system is not fully understood; however, there is evidence that the effects depend on basal dopamine tone (Thomas et al., 2008). Auto-oxidation of dopamine results in the production of reactive oxygen and nitrogen species, and free-radical formation is elevated following methamphetamine administration (Riddle et al., 2006); preventing this oxidation blocks methamphetamine-induced reductions in DAT and tissue-dopamine content (Imam et al., 1999; Fukami et al., 2004; Hashimoto et al., 2004). It is not known, however, whether free-radical formation contributes to the molecular changes elicited by the slow, escalating dose administration regimen reported here.

Striatal D₂-like receptor availability increased in several of the saline-exposed monkeys. As previous studies have reported greater D₂-like receptor levels in rodents exposed to chronic mild stress (Yaroslavsky and Tejani-Butt; Lucas et al., 2007), this effect may be a consequence of multiple, daily control injections, or it could be due to the simple passage of time in the study. In either case, these results emphasize the importance of a true control group.

Effects on Inhibitory Control: Methamphetamine exposure resulted in behavioral deficits that were restricted to the reversal phase of the task: when the contingencies of the task reversed, methamphetamine-treated monkeys required significantly more trials to reach the performance criterion. Similar deficits have been found in human methamphetamine users (Ghahremani et al., 2011) and in rats exposed to multiple, high doses of methamphetamine (Izquierdo et al., 2010). This impairment was most robust during the assessment conducted 3-weeks into the dosing regimen because methamphetamine impaired inhibitory control processes in all monkeys. Because no group differences were detected in the ability of monkeys to acquire a novel stimulus-reward association, methamphetamine exposure appears to impair the ability to recruit the additional cognitive resources that are required when contingencies change in the reversal phase. Unlike previously reported long-

lasting effects of cocaine (Jentsch et al., 2002), the methamphetamine-induced reversal impairment was transient, present only during early withdrawal from methamphetamine. This discrepancy may be due to the extensive pre-training the current subjects received on the task, as the circuitry underlying reversal learning is known to change as a function of experience (Boulougouris and Robbins, 2009; Rygula et al., 2010). Thus, the relatively higher degree of training conducted in this study may have resulted in task performance being governed by an increasingly distributed and/or robust array of neural systems, which in turn may have obscured our ability to detect enduring changes in inhibitory control processes that have been found in cocaine-exposed animals with limited reversal-learning training (Jentsch et al., 2002; Schoenbaum et al., 2004).

Two additional weeks of methamphetamine exposure did not alter reversal-learning performance consistently in all subjects. The reason for the differences in behavioral response to methamphetamine is unknown. However, within methamphetamine-dependent samples there is a high degree of variability in cognitive control capabilities, even when accounting for lifetime methamphetamine use (Cherner et al., 2010). The heterogeneity of the behavioral impact of methamphetamine in the current set of data, in combination with results from studies of methamphetamine-dependent individuals, suggests that pre-existing factors mediate the biobehavioral effects of methamphetamine. Although these factors have yet to be identified, variation in striatal dopamine tone, prior to drug use, represents one potential candidate. Greater amphetamine-induced dopamine release is associated with the degree of self-reported impulsiveness (Buckholtz et al., 2010a), which itself is a risk factor for the development of addictions (Auger et al., 2010; Ersche et al., 2010). Because the effects of methamphetamine on the dopamine system depend on basal dopaminergic tone (Thomas et al., 2008), individual variability in levels of dopamine may mediate the cognitive response to methamphetamine. Further evidence is needed to determine the validity of this hypothesis and to

identify other potential factors that can account for the individual variability in long-term behavioral response to methamphetamine.

Deviations in D₂-like receptor availability are correlated with changes in positive-feedback sensitivity:

The change in D₂-like receptor availability in the dorsal striatum, but not DAT availability, was correlated with the change in positive-feedback sensitivity. These data, taken with previous evidence (Groman et al., 2011), indicate that the D₂-like receptor system plays a role in modulating sensitivity to positive feedback. The correlations were detected only in the reversal phase, most likely due to the heightened demands this phase of the task places on subjects to rapidly incorporate feedback into subsequent responses. The relationship between D₂-like receptor availability and positive-feedback sensitivity was exhibited by both methamphetamine- and saline-exposed groups, indicating that regardless of the influences acting on D₂-like receptor function, regulation of D₂-like receptor availability is associated with changes in positive-feedback sensitivity.

Individual Differences in the Biobehavioral Response to Methamphetamine and Implications for

Addictions: Methamphetamine treatment reduced DAT availability in all monkeys; however, the same was not true regarding D₂-like receptors. Methamphetamine treatment caused large reductions in D₂-like receptor availability for some monkeys (~30-40%), but had little or no effect in others. In the current study, methamphetamine was administered via an experimenter, rather than by the subject. Although the administration model employed in the current study does not capture individual choices in drug use, it does allow for precise control over the dose and timing of drug administration. Even with these controls in place, the results of the current study indicate that there is a substantial degree of variability in the biobehavioral response to methamphetamine. Therefore, biological or environmental factors, outside the patterns or amount of drug intake, affect the biobehavioral effects of drug experience.

Dysfunction of the D₂-like receptor has been proposed to be a common biochemical correlate of addiction (Volkow et al., 1993; Volkow et al., 2001b; Lee et al., 2009; Johnson and Kenny, 2010), and improving function at these receptors is a treatment strategy of interest (Kosten et al., 2002; Thanos et al., 2004; Thanos et al., 2008). Further, deficits in behavioral flexibility are believed to be a core phenotype of addiction (Jentsch and Taylor, 1999) and to be correlated with the retention of cocaine-dependent individuals in a treatment program (Aharonovich et al., 2006), which itself is one of the best predictors of sobriety (Zhang et al., 2003). Indeed, administration of the D₂-like receptor agonist pramipexole alleviates impairments of reversal learning in stimulant-dependent individuals (Ersche et al., 2011), indicating that enhancing D₂-like receptor function can improve inhibitory control and, may, by proxy, improve abstinence rates.

However, D₂-agonist based treatments have been unsuccessful in improving abstinence rates amongst stimulant-dependent individuals (Handelsman et al., 1997). This failure may be due to the high degree of symptom heterogeneity that exists within addictions, particularly related to inhibitory control. Although some drug-dependent individuals have profound cognitive-control impairments, others perform at levels comparable to that of healthy control subjects (Ersche et al., 2008). Here, we also found heterogeneity in the behavioral impact of methamphetamine. Therefore, D₂-like receptor agonists may have the greatest therapeutic benefit in individuals whose primary behavioral deficit is inhibitory control.

Conclusions:

By combining neuroimaging and behavioral techniques, the current study provides a mechanistic understanding of the emergence of inhibitory-control deficits in drug-dependent populations. Though recent reviews of the literature have called into question the behavioral and neural toxicity associated with methamphetamine (Hart et al., 2011), the current results provide unambiguous

data indicating that a realistic model of methamphetamine experience triggers alterations in dopaminergic transmission that likely culminate in the inhibitory-control deficits associated with the persistent use of stimulants (Ersche et al., 2008; Ghahremani et al., 2011). Specifically, we propose that dysfunction of the D₂-like receptor results in reductions in sensitivity to positive feedback, which behaviorally manifests as the habitual and compulsive phenotype exhibited by drug-dependent individuals. These data provide insight into the neural and behavioral consequences of methamphetamine abuse and have broad implications for understanding the biobehavioral interactions that underlie addictions.

Figures

Figure 3.1: D₂-like receptor availability in the caudate nucleus (A), putamen (B), ventral striatum (C), and ventral mesencephalon (D) in saline-exposed (open circles) and methamphetamine-exposed (closed circles) monkeys at baseline, 2 weeks post-drug exposure, and 7 weeks post-drug exposure. * indicates a significant change from baseline; # indicates a significant change from 2 weeks post-drug exposure. ****p* < 0.001, ####*p* < 0.001. SED, SE of the mean of differences.

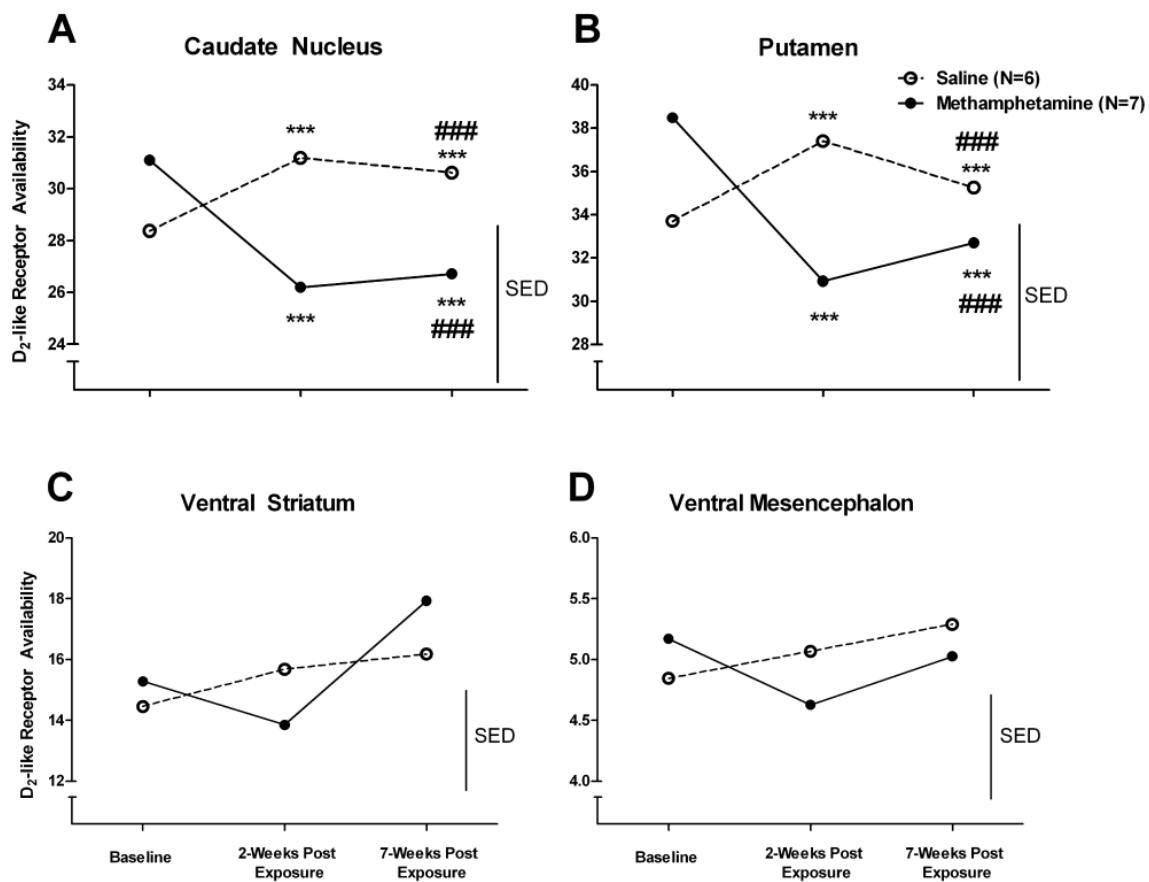


Figure 3.2: DAT availability in the caudate nucleus (A), putamen (B), ventral striatum (C), and ventral mesencephalon (D) in saline-exposed (open circles) and methamphetamine-exposed (closed circles) monkeys at baseline, 2 weeks post-drug exposure, and 7 weeks post-drug exposure. * indicates a significant change from baseline; # indicates a significant change from 2 weeks post-drug exposure. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, #### $p < 0.001$. SED, SE of the mean of differences.

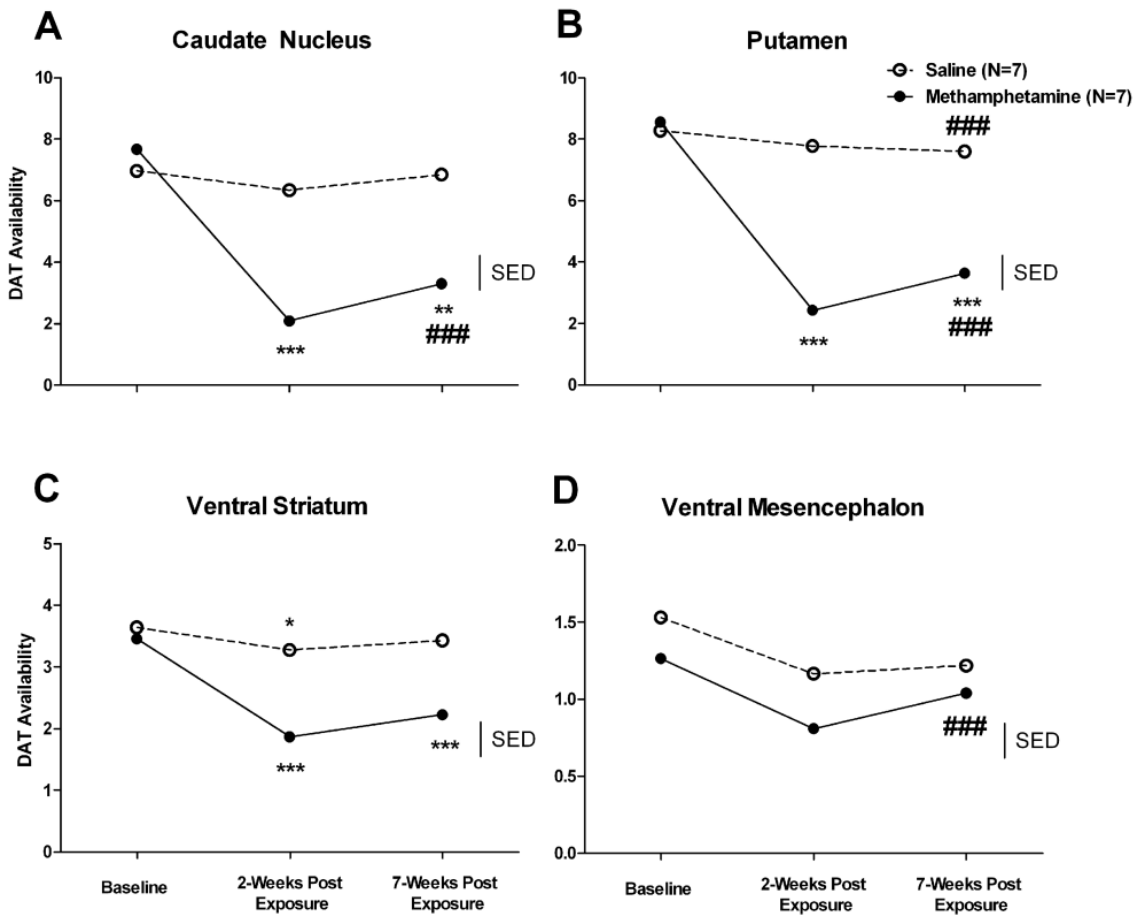


Figure 3.3: The number of trials required to reach criterion in the acquisition (A), retention (B), and reversal (C) phases between saline-exposed (open circles) and methamphetamine-exposed (closed circles) monkeys at baseline, at the 3 week assessment, at the 5 d post-drug exposure test, and at the two high difficulty sessions (1 week post-drug exposure and 3 weeks post-drug exposure). *** $p < 0.001$. SED, SE of the mean of differences.

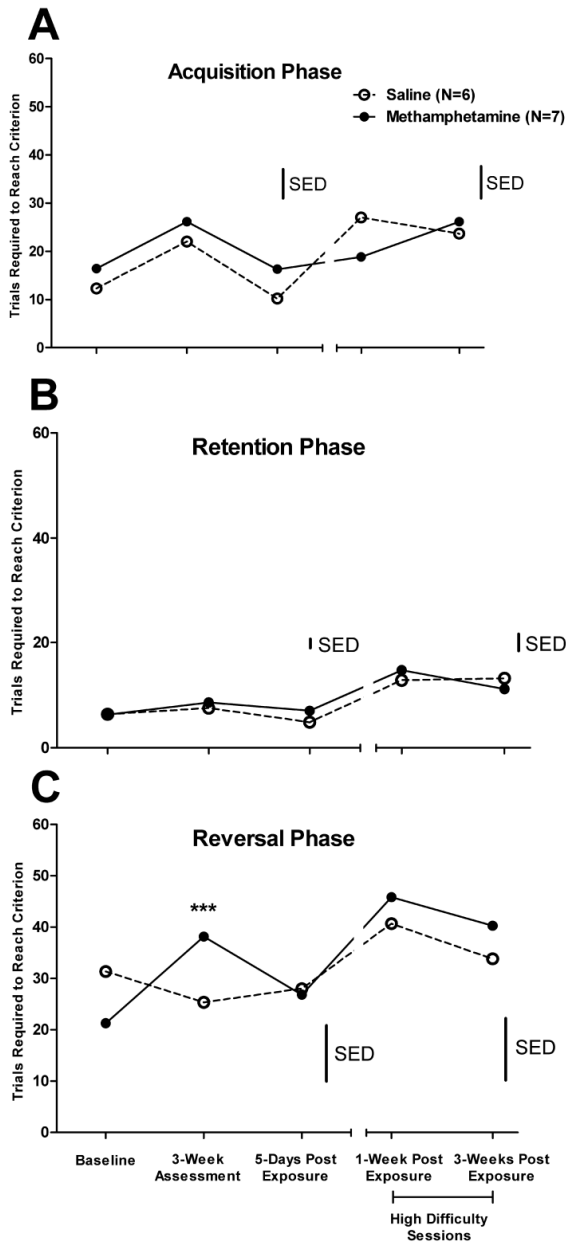
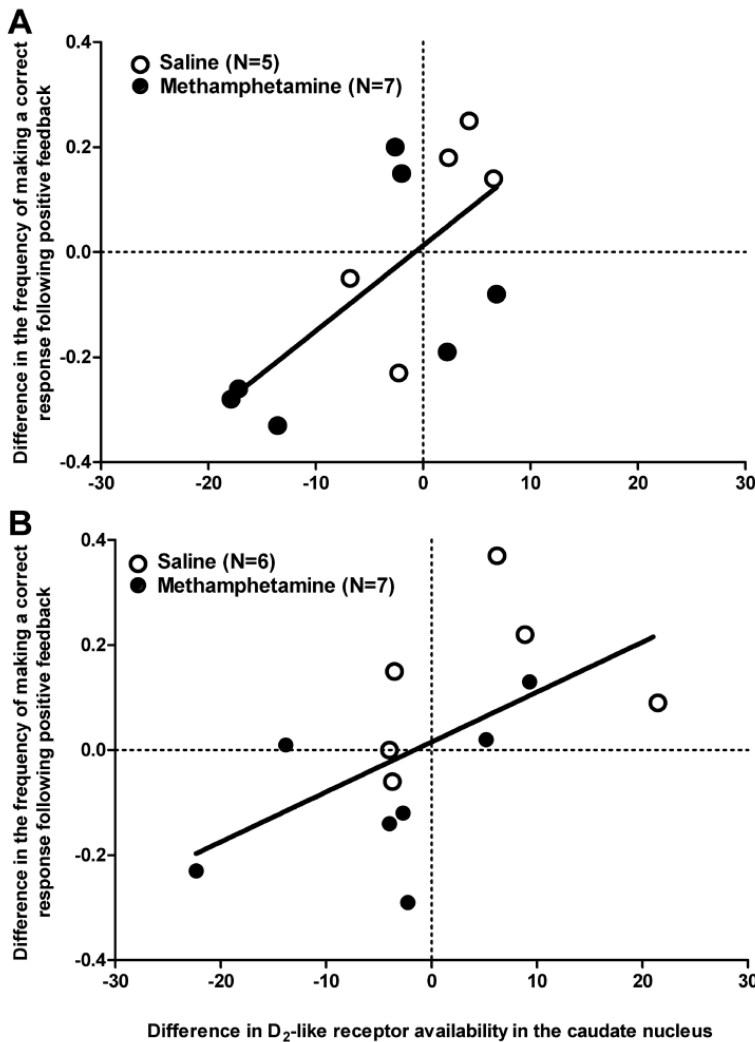


Figure 3.4: The relationship between changes in D₂-like receptor availability in the caudate nucleus and in the ability of monkeys to persist with a correct response following positive feedback in saline-exposed monkeys (open circles) and methamphetamine-exposed monkeys (closed circles). **A**, Compares the change from baseline in D₂-like receptor at the 1 week postexposure scan to change from baseline in positive-feedback sensitivity at the 5 d postexposure assessment. **B**, Compares the change from baseline in D₂-like receptor at the 7 week postexposure scan to change from baseline in positive-feedback sensitivity at the 3 week postexposure assessment.



Tables:

Table 3.1: Dose of methamphetamine (mg/kg) given per day and at each injection during the 31 d dose regimen.

Table 1. Dose of methamphetamine (mg/kg) given per day and at each injection during the 31 d dose regimen

Week 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Injection #1	0.1	0.2	0.3	0.4	0.5	
Week 2						
Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Injection #1	0.25	0.35	0.45	0.55	0.65	
Injection #2	0.25	0.35	0.45	0.55	0.65	
Week 3						
Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
Injection #1	0.35	0.45	0.55	0.65		
Injection #2	0.45	0.55	0.65	0.75		
Injection #3	0.50	0.60	0.70	0.80		
Week 4						
Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27
Injection #1	0.6	0.7	0.8	0.9	1.0	
Injection #2	0.6	0.7	0.8	0.9	1.0	
Injection #3	0.6	0.7	0.8	0.9	1.0	
Injection #4	0.6	0.7	0.8	0.9	1.0	
Week 5						
Day 28	Day 29	Day 30	Day 31			
Injection #1	1.0	1.0	1.0			
Injection #2	1.0	1.0	1.0			
Injection #3	1.0	1.0	1.0			
Injection #4	1.0	1.0	1.0			

Saline-exposed monkeys received injections of saline using the same regimen.

Chapter 4

Naturally-occurring variation in dopamine D₂-like receptor availability reflects differences in D₂-like receptor density and function: implications for addiction and inhibitory control mechanisms

Summary

The use of positron emission tomography (PET) in neuroimaging research has made possible crucial insights into the pathophysiology of addiction. Amongst the important discoveries made possible using this method is the identification of low dopamine D₂-like receptor availability, leading to altered inhibitory control processes, as both an influence on and a consequence of addiction-related behaviors. These differences in D₂-like receptor availability can reflect multiple biological phenomena, including receptor density in the region of interest, the intrasynaptic concentration of endogenous competitive factors (including dopamine, itself) and receptor affinity, as well as differences in functional and non-functional receptor pools. For all these reasons, PET, while noninvasive and suitable for longitudinal studies in humans and animals, lacks biological resolution. Here, we use neuroimaging, pharmacological, behavioral and biochemical methods to delineate the specific biological adaptations that underlie individual differences in dopamine D₂-like receptor availability that are related to inhibitory control. *In vivo* measures of D₂-like, but not D₁-like, receptor availability acquired using PET, were correlated positively with competency in a test of inhibitory control. Dopamine D₂-like receptor availability was also positively correlated with eye blink rate (EBR; a known dopamine-sensitive motor response) and D₂-agonist induced increases in EBR, as well as with D₂ receptor concentration determined in brain homogenates. Our results show that that spontaneous eye blink rate represents a high-fidelity, non-invasive behavioral marker of D₂-like receptor function. More importantly, perhaps, our studies provide the first direct evidence demonstrating that differences in D₂-like receptor availability detected with PET

that are relevant to inhibitory control loss and susceptibility for addictions reflect differences in D₂-like receptor density that cannot be accounted for by differences in dopaminergic tone

Introduction

Extensive research supports the notion that experience with drugs of abuse elicits neuroadaptions that contribute directly to drug-induced changes in behavioral responses to the drug (Reith, 1986; Czoty et al., 2010), cognitive and executive functions (Jentsch et al., 2002; Schoenbaum et al., 2004; Schoenbaum and Setlow, 2005), incentive motivation (Taylor and Jentsch, 2001; Olausson et al., 2004; Berridge, 2012), affect and mood (Ahmed and Koob, 1998). These neuroadaptations include persistent reductions in dopamine synthesis, synaptic dopamine levels and alterations in pre- and post-synaptic dopamine receptor density and function (Melega et al., 1997; Sabol et al., 2001; Melega et al., 2008); many of these observations hold in both drug-dependent individuals and animals with chronic exposure to these drugs (Volkow et al., 1993; Volkow et al., 2001b; Nader et al., 2006; Lee et al., 2009; Groman et al., 2012). Furthermore, some of these neurochemical changes within the dopamine system are directly associated with behavioral characteristics that predict disability (Nader et al., 2006; Dalley et al., 2007; Groman et al., 2011) and response to treatment and abstinence (Martinez et al., 2011; Wang et al., 2012), demonstrating their importance as clinically-relevant biomarkers.

Amongst the dopaminergic neuroadaptations demonstrated in both human drug users and animal models of addiction are signs of low dopamine D₂-like receptor number or function; low D₂-like receptor availability has been argued to be a biochemical phenotype common to many forms of behavioral addictions (Groman and Jentsch, 2011b). Specifically, low D₂-like receptor availability, measured with positron emission tomography (PET) is present in cocaine- and methamphetamine-dependent individuals (Volkow et al., 1993; Volkow et al., 2001b; Lee et al., 2009) as well as in obese, opiate- and nicotine-dependent individuals (Fehr et al., 2008; Volkow et al., 2008b; Zijlstra et al., 2008) compared to controls. Studies in animal subjects indicate that reductions in D₂-like receptor availability occur as a result of chronic exposure to drugs (Nader et al., 2006; Groman et al., 2012). However, individual, naturally-occurring differences in D₂-like receptor availability, prior to any drug use, vary with

inhibitory control abilities (Groman et al., 2011) and predict future drug-taking behaviors (Dalley et al., 2007; Michaelides et al., 2012), suggesting that pre-existing D₂-like dysfunction may influence the susceptibility of individuals to develop addictive-like behaviors, perhaps through eroding the ability to exert control over drug-seeking and –taking (Groman and Jentsch, 2013b). These collective data, therefore, support the notion that low dopamine D₂ receptor availability, possibly compromising dopamine D₂-mediated transmission, is a common biomarker of susceptibility for, and development of, addictions – possibly through a causal loss of inhibitory control ability (Groman and Jentsch, 2011b).

Positron emission tomography (PET) is the most commonly used technique for *in vivo* quantification of dopamine D₂-like receptors and has been implemented in humans, non-human primates and rodents, proving to be a highly-translational tool. Although differences in receptor availability measurements have been assumed to reflect differences in receptor density, PET measurements almost certainly can be influenced by multiple changes in receptor number and function that limit its biological resolution. Specifically, differences in D₂-like receptor availability measured with PET could result from a) differences in endogenous dopamine levels that compete with the radiotracer for the receptor, b) differences in nonfunctional receptor pools accessible to the radiotracer or c) as presumed, differences in the complement of biologically functional receptors. Typical PET assessment sessions involving a competitive radiotracer would be unable to disentangle each of these biological factors, even though the degree to which one or the other influence the overall availability measure changes the hypothesis about how this measure predicts underlying alterations in neurotransmission and, potentially, treatment.

The goal of the current study was to determine the underlying biological mechanisms that contributed to behaviorally-relevant individual differences in brain dopamine D₂-like receptor availability. To do this, we selected 10 monkeys that varied, naturally, in their intrinsic ability to inhibit in

appropriate responses, as measured by a reversal learning test; these behavioral differences were then related to PET measurements of dopamine D₁-like and D₂-like receptors. We then examined whether PET measures of D₂ receptor availability represented a functional pool of receptors by quantifying whether receptor availability associated with dopamine agonist-induced changes in eye blink rate, a motor response known to be sensitive to dopamine receptor function (Elsworth et al., 1991; Taylor et al., 1999). Following the *in vivo* assessments, dopamine levels and [³H]spiperone binding were assessed in tissue homogenates *ex vivo*. Based on our previous results, we expected natural variation in D₂-like receptor availability to relate to individual differences in reversal learning performance and positive-feedback sensitivity. Further, we hypothesized that D₂-like receptor availability would be associated with D₂-agonist induced changes in eye blink rate and D₂-like receptor density, but not levels of dopamine.

Methods

Subjects: 10 adult, male vervet monkeys born to different parents in the same birth cohort (2002) were included in the current study. Subjects were socially-housed at the UCLA Vervet Research Colony, where they were born, until they were relocated to UCLA. From this point forward, the subjects were peer-housed in large (~25'x12'x8') semi-outdoor enclosures fitted with toys, exercise materials and appropriate environmental enrichment. Subjects had unlimited access to water and were provided twice daily portions of standard monkey chow (Teklad) that exceeded their caloric requirements. Fruit and vegetable enrichment was provided daily. Food intake was never reduced to encourage participation in behavioral testing.

All monkeys were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the University of California, Los Angeles, Chancellor's Animal Research Committee.

Drugs: The D₂-like agonist (+) 4-propyl-9-hydroxynaphthoxazine (PHNO; ABX advanced biochemical compounds, Germany) and the D₁-like agonist (1R,3S) 3-(1'-adamantyl)-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran hydrochloride (A77636; Sigma-Aldrich, St Louis, MO) were dissolved in 0.9% saline to generate the following concentrations: 0.5, 0.05, and 0.005 mg/ml. The selective D₂ antagonist L 741626 (Sigma-Aldrich) was dissolved in 10% DMSO, 10% TWEEN in sterile water to generate the following concentrations: 3 mg/ml and 0.3 mg/ml. The selective D₃ antagonist NGB 2904 (graciously donated by Dr. Robert Roth) was dissolved in 25% (2-Hydroxypropyl)-Beta-Cyclodextrin (Sigma-Aldrich) in sterile water to generate the following concentrations: 7.5 mg/ml and 0.75 mg/ml. All drugs were administered at 0.1 ml/kg intramuscularly.

Discrimination acquisition, retention and reversal learning: Monkeys were assessed for their ability to acquire, retain and reverse visual discrimination problems, using procedures similar to those previously described (Groman et al., 2013). Subjects were trained to enter into a metal tunnel that was fitted with a modified Wisconsin General Testing Apparatus. This apparatus was equipped with an opaque screen that could be raised or lowered; when it was raised, three equally spaced opaque boxes were visible to, and could be accessed by, the monkey. Each box was fitted with a hinged lid that allowed for small pieces of a food reward (i.e., a piece of banana, apple, or grape) to be concealed within the box.

Testing sessions began when the opaque screen was raised to present the three boxes (each fitted with a unique visual stimulus) to the monkey. At the inception of a new training session, one of the three visual stimuli was deterministically associated with food reward, while the others were not. Monkeys were only allowed to open one box per trial. A trial ended after a correct choice (opening the box containing the food reward), an incorrect choice (opening an empty box), or an omission (no response for 30 sec) occurred. The position of the visual stimuli/food rewards was pseudorandomly varied across trials. Subjects received up to 30 trials per day.

Initially, subjects were trained to acquire seven consecutive discrimination problems, each involving 3 novel visual stimuli. Subjects were required to learn which one of the three stimuli was associated with food reward by achieving a performance criterion (the first 3 discrimination problems used a criterion of 7 correct choices within 10 consecutive trials; the last 4 discrimination problems used a criterion of 8 correct choices within 10 consecutive trials). If the performance criterion was not achieved in 30 trials, the session was terminated and the monkey was returned to the social enclosure. The same discrimination problem was presented the following day(s) until the performance criterion was met.

After completing the seven discrimination-acquisition problems, monkeys were then trained to acquire, retain, and reverse novel discrimination problems. The first phase of each discrimination problem was an acquisition phase that was identical to the acquisition-only training the monkeys had previously received. Once the same performance criterion had been met (eight correct choices within 10 consecutive trials), the session was terminated and the monkey was returned to the social enclosure.

Twenty-four hours later, the ability of subjects to remember the stimulus-reward association from the day before was assessed in a retention phase, wherein the stimulus-reward contingencies remained unchanged relative to the previous training day. This phase continued until subjects met the performance criterion of four correct responses in five consecutive trials. Immediately after reaching the criterion in the retention phase, the reversal phase of the discrimination problem began; there were no environmental events that cued this transition, other than a change in reinforcement contingencies. In the reversal phase, the stimulus that was previously rewarded was no longer rewarded, and one of the two previously unrewarded stimuli was now rewarded. The reversal phase persisted until monkeys achieved criterion (8 correct choices in 10 consecutive trials) or until 30 trials had been completed, whichever occurred first. If monkeys did not reach the performance criterion within 30 trials, the reversal phase continued the following day(s) until the performance criterion was met.

After completing three sets of acquisition/retention/reversal problems, two additional, theoretically more difficult, tests were conducted in which the performance criterion was raised to eighteen correct responses within twenty consecutive trials for both the acquisition and reversal phases and eight correct responses within ten consecutive trials for the retention phase.

The primary dependent measures were the total number of trials required to reach criterion and the number of correct responses made in the acquisition, retention, and reversal phases. For the

reversal phase, the total number of responses directed at the previously rewarded stimulus (a perseverative response) and the never rewarded stimulus (a neutral response) were also recorded.

Not all monkeys completed all the stages of testing due to environmental circumstances or veterinary interventions. However, acquisition, retention and reversal performance data were collected in nine of the ten subjects for the last discrimination problem (one subject was excluded due to an injury that occurred during testing); behavioral data collected during this final stage of discrimination training was the primary outcome used in the current study.

Feedback sensitivity measures: The sensitivity of individuals to positive and negative feedback was determined by an analysis of choice behavior on a trial-by-trial basis during the reversal phase, as previously described (Groman et al., 2011; Groman et al., 2012). Trials were categorized based on whether the subject experienced positive or negative feedback on the preceding trial. We then calculated the probability that after experiencing positive feedback, a subject would make either: (1) another correct response, (2) a response directed to the stimulus that was previously rewarded, or (3) a response directed at the stimulus that was never rewarded. Sensitivity of individuals to negative feedback was assessed by determining the probability that after experiencing negative feedback subjects made either: (1) the same incorrect response or (2) a response directed at a different stimulus, regardless of whether this response was correct or incorrect.

PET Scanning Procedures: PET scanning was performed using [¹⁸F]fallypride and [¹¹C]NNC-112 as radioligands for measurements of D₂-like and D₁-like receptor availability, respectively. PET scans were collected at a time point less than 3 months from the final reversal learning test reported here. On PET scanning days, monkeys received an intramuscular injection of ketamine hydrochloride (10mg/kg) and glycopyrrolate (0.01 mg/kg). After monkeys were sedated, an endotracheal tube was placed to provide 2-3% isoflurane anesthesia (in 100% O₂) for the duration of the procedure. Vital signs (heart rate,

respiratory rate, oxygen saturation and temperature) were monitored and recorded every 15 min throughout the scan. A tail-vein catheter was placed and the monkey positioned on the scanning bed such that the imaging planes were parallel to the orbitomeatal line and the top of the head at the front of the field of view. A 20 min ^{57}Co transmission scan was acquired before administration of the radioligand for attenuation correction. All subjects received a bolus injection of [^{11}C]NNC-112 (0.3 mCi/kg) followed by a 5-ml saline flush, and data were acquired for 120 min. When radioactivity had fallen to baseline levels (~ 3 h after administration of [^{11}C]NNC-112), a bolus injection of [^{18}F]fallypride was delivered (0.3 mCi/kg), followed by a 5-ml saline flush, and data acquired for 180 min. After the scan, gas anesthesia was removed, and the monkey was allowed to recover overnight before being returned to its social enclosure.

Due to radioligand synthesis problems, four of ten monkeys were scanned using two separate sessions (each involving one of the radioligands), with an inter-scan period of 30-60 days.

Acquisition of magnetic resonance images: MR images for the subjects were collected as a part of a larger study (Fears et al., 2009). Nine T1-weighted volumes with three-dimensional MPRAGE images (TR = 1900 ms, TE = 4.38 ms, FOV = 96 mm, flip angle 15° , voxel size 0.5 mm, 248 slices, slice thickness 0.5 mm) were collected using 8-channel, high-resolution knee-array coil (Invivo) in a 1.5T Siemens scanner. Individual images were aligned to each other using Statistical Parametric Mapping 5 (Institute of Neurology, University College London, London, UK), averaged together, and resliced according to a previously developed MR template (Groman et al., 2011).

Eye blink video recording: Approximately two years after the PET scans, eye blink rate was measured in the same subjects. Monkeys were video recorded while sitting in a modified metal tunnel equipped with two transparent sliding windows. Two high-definition digital video cameras (Bloggie, Sony) were positioned directly in front of the transparent windows which allowed 180 degree visibility of the

monkey. Recording began simultaneously on the two video cameras and continued for 60-90 min, depending upon whether the session involved administration of a drug, or not.

Two 60-min video recordings separated by 85 days were first collected in each subject in order to determine the reliability of spontaneously-occurring eye blinks within and between these sessions (referred to as “SEBR Session 1” and “SEBR Session 2”, respectively). Prior to beginning the pharmacological portion of the study, monkeys received four saline injections (two injections per week) while the eyes of the monkey were recorded in the metal tunnel in order to habituate them to the brief intramuscular injections.

The pharmacological portion of the study was completed across a ten-week period, during which baseline and post-drug administration video recordings were collected once per week in each subject. Following 30 min of baseline recording, monkeys received an intramuscular injection of the D₁-like agonist (0.005, 0.05, and 0.5 mg/kg), D₂-like agonist (0.005, 0.05, and 0.5 mg/kg) or saline, and were recorded for an additional 60 min. Because dopamine receptor agonists are known to rapidly sensitize receptors, the order of drug administration was not fully randomized. Rather, the order of drug administration alternated between the D₁-like and D₂-like agonist each recording session, with a subject receiving one dose of the D₂-like agonist the first week, one dose of the D₁-like agonist the second week, one dose of the D₂-like agonist the third week, etc. The order of the dose for each agonist was randomly assigned using a Latin Square design. In addition to these drug sessions, four recording sessions were also collected in which saline only was administered following the 30-min baseline recording: one conducted the week prior to starting the dopamine agonist regimen, one conducted between the first and fourth dopamine agonist session (randomly assigned), one conducted between the fifth and tenth dopamine agonist session (randomly assigned), and one conducted after completing the ten week experiment.

Some of the drug challenge sessions were repeated because the visibility of the subject's eyes was extremely low, hindering our ability to measure eye blink rate accurately. These sessions were collected approximately 4 months after completing the 10-week drug schedule, after baseline eye blink rates had been re-determined.

Next, eye blinks were recorded in monkeys following administration of the D₂ preferring antagonist (L741626; 0.3 and 0.03 mg/kg) or the D₃ preferring antagonist (NGB 2904; 0.75 and 0.075 mg/kg) in order to determine the relative contribution of these receptor subtypes to D₂-like receptor availability measurements. The analysis of these data is currently on-going and not included in the current experimental results.

Collection of tissue for ex vivo measures: Approximately 8 months after completion of the eye blink measurements, monkeys were chemically restrained with ketamine hydrochloride (10mg/kg, i.m.) and heavily sedated with euthasol (30 mg/kg). Following loss of the corneal reflex, monkeys were transcardially perfused with ice-cold saline for 10 min; brains were then removed and tissue punches collected for monoamine quantification on a cold platform (Raymond Lamb). The remaining tissue slices were snap frozen in isopentane and stored in a -80 freezer until regional dissection of the striatum was conducted for the radioligand binding assays.

Quantification of monoamine and monoamine metabolites: Monoamine and metabolite levels in the caudate nucleus, putamen and ventral striatum were measured with high pressure liquid chromatography using procedures previously described (Jentsch et al., 1997).

Preparation of membrane homogenates for [³H]spiperone binding assay: Striatal tissue was regionally dissected from the tissue slices described above. Following a slow defrost of the tissue slices, the caudate nucleus, putamen and nucleus accumbens were dissected out manually on a cold platform (Raymond Lamb), weighed and placed in a -80°C freezer until processed. Tissue was homogenized in 20

volumes of assay buffer (50 mM Tris HCl, 50 mM Tris Base, 5 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, pH to 7.4 at 23 °C), using a tissue sonicator (Sonic Dismembrator Model 100, Fisher Scientific). Tissue homogenate were centrifuged twice at 15000 RCF for 15 min, resuspending the pellet each time in 20 volumes of assay buffer. The final tissue pellet was suspended in assay buffer to yield a final tissue concentration of 1 mg original wet tissue weight (o.w.w.) per ml of buffer.

[³H]Spiperone binding assays: Binding assays were performed in duplicates in disposable polystyrene tubes in a final volume of 0.5 ml using procedures previously described (Levant, 2007). A ten-point saturation curve was conducted, with the highest final concentration of [³H]spiperone at approximately 5 nM. Binding was initiated by the addition of membrane homogenate (3 mg/ml o.w.w.) to [³H]spiperone. Nonspecific binding was defined in the presence of 5µM (+)-butaclamol. Samples were incubated for 90 min at 27 °C, and the reaction was terminated by separation of the free from the bound radioligand by rapid vacuum filtration over Whatman GF/B filters (pre-soaked in 0.5% PEI at room temperature for 90 min) using a Brandel Cell Harvester. Filters were washed three times with 2 ml of ice-cold wash buffer (50 mM Tris-HCl and Tris-Base, pH 7.4 at 27 °C) and allowed to dry before being placed in 2 ml of liquid scintillation fluid (Ultima Gold, Perkin Elmer). Filters were soaked for no less than 6 hr, but no more than 24 hr, in the scintillation fluid before being counted in a liquid scintillation counter (Beckman LS 6500, Beckman Coulter). Specific [³H]spiperone binding was determined by subtracting nonspecific binding from total binding and was expressed as femtomoles per milligrams of protein of wet tissue weight. Due to technical complications, [³H]spiperone binding was only determined for the caudate nucleus; [³H]spiperone binding in the putamen and nucleus accumbens will be completed in the future.

Data processing:

Reconstruction of PET images: Three-dimensional sinogram files were created by binning the data into 21 frames (four 60-s frames, three 120-s frames, six 300-s frames, and eight 600-s frames) for [¹¹C]NNC-112 sinograms and 33 frames (six 30-s frames, seven 60-s frames, five 120-s frames, four 300-s frames, nine 600-s frames, one 1200-s frame and one 1800-s frame) for [¹⁸F]fallypride sinograms. We applied a previously validated algorithm to the list-mode data from the transmission scan to generate attenuation maps (Vandervoort and Sossi, 2008). Following construction of the attenuation maps, emission list-mode files were reconstructed using Fourier rebinning and filtered back projection, corrected for normalization, dead time, scatter and attenuation with software provided by Concorde Microsystems (microPET manager version 2.4.1.1, Siemens). The resultant images had voxel dimensions of 0.949 x 0.949 x 1.212 mm³ and matrix dimensions of 128 x 128 x 63.

PET data processing: Using FSL View (FMRIB's Software Library v4) the following ROI's were drawn on the structural MR image of each subject by a single experimenter: the caudate nucleus, putamen, ventral striatum and cerebellum.

Reconstructed PET images were corrected for motion, and were co-registered to the subject's MR image using the PFUS module within PMOD (version 3.15; PMOD Technologies). Using the putamen and cerebellum ROIs, activity was extracted from the coregistered PET images and imported into the kinetic analysis program within PMOD (PKIN). The time-activity curves were fit using the simple reference tissue model (SRTM) to provide an estimate of the k₂' value. Parametric binding maps, showing binding potential (BP_{ND}), were generated for each subject in PXMOT using the SRTM2 model with the fixed k₂' value obtained from analysis of the time-activity curves. Activity extracted from the putamen and cerebellum was entered as references for high and low activity areas, respectively. To conduct the voxelwise statistical analysis, each subject's MR image was aligned to a previously developed MR

template (Groman et al., 2011) using FSL FLIRT and the resultant transformation matrix applied to the BP_{ND} maps. A 2FWHM Gaussian smoothing kernel was then applied to the maps.

Calculating spontaneous eye blink rate: The video recordings of eye blinks were manually scored by experimentally-blinded raters using a program developed in-house. The program allowed raters to time-stamp the occurrence of a blink (defined as a full opposition of the eye lid that lasted less than a second) and the visibility of the subject's eyes (as variation in visibility can directly influence eye blink rate) while simultaneously viewing 2 video streams from the cameras positioned at 90 degrees from one and other. Video raters were trained to an inter-rater reliability of at least 90% before beginning analysis of the current videos. Data was binned into 5-min blocks, and eye blink rate within each block was computed as the total number of blinks per total visibility time. If visibility of the monkey was less than 2 min of the 5 min block, data from that block were excluded from the analysis.

Calculating pharmacological changes in eye blink rate: Average eye blink rate for the 30 min before drug (or vehicle) administration and 60 min following administration were the primary dependent measures. Inspection of the data indicated that there were temporal changes in eye blink rate following administration of drug that may not have been captured with eye blink measurements averaged over the entire 60 min session. Eye blink rate has been found to increase rapidly following administration of dopamine agonists (Elsworth et al., 1991; Kleven and Koek, 1996), returning to baseline levels 30-40 min following drug administration. To characterize drug-induced changes in eye blink rate more fully, we determined the maximal and minimal eye blink rate before and after drug (or vehicle) administration, as well as the average response across the entire session.

Eye blink rate for the 30 min prior to drug (or vehicle) administration was subtracted from the eye blink rate for the 60 min following drug. To verify that the pharmacological effects were specific to the drug (versus stress of the injection), change in eye blink rate from baseline following administration

of saline was subtracted from the change in eye blink rate from baseline following administration of drug. These calculations were done for the average eye blink rate, maximal eye blink rate and minimal eye blink rate.

Statistical analyses: Statistical analyses were primarily conducted in SPSS (v 15.0). Reliability of eye blink counting and visibility estimates were analyzed with the average intra-class correlation for the following measures: total eye blinks per video per subject, total visibility per video per subject, and average eye blink rate per video per subject. The statistical dependency between eye blink rate (both spontaneous and drug-induced changes) and ROI-based measurements of dopamine receptor availability and/or visual discrimination performance of monkeys was examined with Pearson's product-moment correlation coefficients. Based on the results of this analysis (described below), the anatomical specificity of the relationship between D₂-like receptor availability and spontaneous eye blink rate was examined by regressing eye blink rate (both spontaneous and drug-induced changes) against the D₁-like and D₂-like binding potential maps (described above) using RANDOMISE with variance smoothing of 2 mm (FWHM), confined to the search volume of the striatum (bilateral caudate, putamen and ventral striatum). Ten thousand randomization runs were performed, and TFCE was used to detect clusters of significant correlation. Statistical maps were thresholded at $p < 0.05$ (one-tailed) and corrected for the entire search volume of the striatum. All other relationships were assessed with the Pearson's product-moment correlation coefficients.

Results

Acquisition, retention and reversal performance: On average, monkeys required 79.8 +/- 16.1 (mean +/- SEM) trials to reach criterion in the acquisition phase, 10.9 +/- 1.4 (mean +/- SEM) trials to reach criterion in the retention phase and 94.2 +/- 13.5 (mean +/- SEM) trials to reach criterion in the reversal phase. An analyses of the type of error made during reversal learning indicated that the probability of making a response to the previously rewarded stimulus was significantly greater than that of making a response to the never rewarded stimulus ($t_9=8.19$; $p<0.001$).

Dopaminergic correlates of acquisition, retention and reversal behavior: A previous study involving a separate cohort of vervet monkeys indicated that striatal D₂-like receptor availability was positively correlated with individual competency during the reversal learning phase (Groman et al., 2011). To determine if this relationship was also present in this subset of monkeys, we tested whether D₂-like receptor availability in the caudate nucleus, putamen and ventral striatum was correlated with the number of trials required to reach criterion in the acquisition, retention and reversal phases. Consistent with our previous findings, D₂-like receptor availability was not significantly correlated with the ability of subjects to acquire or remember the visual discrimination (all p 's>0.25). As in our previous study, the ability to inhibit or update behavior in the reversal learning testing was positively correlated with dopamine D₂ receptor availability; however, unlike our previous findings, the effect was observed in the ventral, but not dorsal, striatal regions (ventral striatum: $r_9=-0.70$; $p=0.02$; caudate nucleus: $r_{10}=-0.39$; $p=0.26$; putamen: $r_9=-0.10$; $p=0.80$; Figure 1A).

Previously, we showed that D₂-like receptor availability was correlated with the behavioral sensitivity of monkeys to positive, but not negative feedback (Groman et al., 2011). To determine if this was also true in the current sample, the statistical relationship between D₂-like receptor availability and feedback sensitivity measurements was examined. Ventral striatal D₂-like receptor availability was correlated with positive-feedback sensitivity (probability of monkeys persisting with a correct response

following positive feedback: $r_9=0.76$, $p=0.03$, Figure 1B; probability of monkeys shifting their response back to the previously rewarded stimulus following positive feedback: $r_9=-0.69$, $p=0.04$, Figure 1C), but not with negative-feedback sensitivity (all p 's>0.56), recapitulating our previous results.

Next, the relationship between D₁-like receptor availability and performance of monkeys in the acquisition, retention and reversal phase was examined. D₁-like receptor availability was not correlated with the ability of monkeys to acquire (all p 's>0.13), retain (all p 's>0.33) or reverse a visual discrimination problem (all p 's>0.36; data not shown). Further, striatal D₁-like receptor availability did not correlate with the sensitivity of individuals to positive or negative feedback (all p 's>0.14), providing further support for the selectivity of the relationship between D₂-like receptors and positive-feedback sensitivity. D₁-like and D₂-like receptors were positively correlated in the putamen ($r_{10}=0.63$; $p=0.04$), but not the in the caudate nucleus or ventral striatum (all p 's>0.15).

Characteristics of spontaneous eye blink rate: We next set to test whether individual differences in brain dopamine receptor availability were reflected in the expression of a dopamine-sensitive spontaneous motor behavior: the eye blink rate. Average eye blink rates for the two spontaneous eye blink assessments (referred to as SEBR session 1 and session 2) were 4.56 +/- 0.70 blinks per min and 3.42 +/- 0.45 blinks per min (mean +/- SEM), respectively. Eye blink rate was extremely reliable within a recording session (Cronbach's alpha SEBR Session 1: 0.96, Figure 1A; Cronbach's alpha SEBR Session 2: 0.90), and average eye blink rate between these sessions was highly correlated ($r_{10}=0.79$; $p=0.006$; Figure 1B), indicating that eye blink rate is stable and trait-like over extended periods of time.

The statistical relationship between average eye blink rate and dopamine receptor availability was examined next. Average eye blink rate from the first recording session was positively correlated with the ROI-based measurements of D₂-like receptor availability in the caudate nucleus ($r_{10}=0.65$; $p=0.04$; Figure 2A) and ventral striatum ($r_{10}=0.74$; $p=0.02$; Figure 2C), with a similar, albeit non-

significant trend for a relationship involving the putamen ($r_{10}=0.58$; $p=0.07$; Figure 2B). In contrast, average eye blink rate did not correlate with D_1 -like receptor availability in any of the striatal regions examined (caudate nucleus: $r_{10}=0.36$, $p=0.31$; putamen: $r_{10}=0.04$, $p=0.91$; ventral striatum: $r_{10}=0.07$, $p=0.83$; Figure 2A-C). Average eye blink rate was then regressed against the D_2 -like binding potential maps to determine the distribution of this relationship within the striatum. Consistent with the ROI analysis, average eye blink rate was positively correlated with D_2 -like receptor availability throughout the striatum (Figure 2D-F).

Three multiple linear regression analyses were conducted using a forced entry model with two steps; for these analyses, D_1 -like and D_2 -like receptor availability, measured in the caudate nucleus, putamen or ventral striatum, was regressed against spontaneous eye blink rate. Inclusion of D_1 -like receptor availability did not explain a significantly greater amount of variance in spontaneous eye blink rate than did D_2 -like receptor availability (R square change for caudate nucleus: 0.03, putamen: 0.17, ventral striatum 0.01; all F 's<2.54). However, the beta coefficients for D_1 -like receptor availability, unlike those for D_2 -like receptor availability, were negative suggesting that D_1 -like receptor availability may exert a small, negative influence on spontaneous eye blink rate.

Pharmacological perturbations of eye blink rate: We next set to test the notion that the total receptor population measured with PET was functionally-active and, therefore, predictive of response to a dopamine receptor agonist challenge. The average change in eye blink rate across the three doses of the dopamine D_2 agonist, PHNO, was not significant (average eye blink rate: $F_{2,8}=1.29$; $p=0.33$; maximal eye blink rate: $F_{2,8}=1.92$; $p=0.21$; minimal eye blink rate: $F_{2,8}=0.58$; $p=0.59$). The same non-significant effect was detected across the three doses of A77636 (average eye blink rate: $F_{2,8}=2.90$; $p=0.11$; maximal eye blink rate: $F_{2,8}=2.07$; $p=0.19$; minimal eye blink rate: $F_{2,8}=2.32$; $p=0.16$). Although the lack of a dose-dependent change in eye blink rate was somewhat surprising, the lack of an effect at a group level

appeared to be because of the high degree of variability between individual subjects in response to the agonists.

We therefore examined whether the change in eye blink rate estimates following administration of the dopamine receptor agonists was correlated against striatal dopamine receptor availability estimates. D₂-like receptor availability in the ventral striatum was positively correlated with the change in average eye blink rate in response to the lowest, threshold dose, of PHNO (0.005 mg/kg; $r_{10}=0.75$; $p=0.01$), while D₂-like receptor availability in the putamen was positively correlated with the change in average eye blink rate following administration of the 0.05 mg/kg dose of PHNO ($r_{10}=0.65$; $p=0.04$). D₂-like receptor availability was not correlated with the change in average eye blink rate following administration of the 0.5 mg/kg dose of PHNO (all p 's>0.35). In contrast, the change in average eye blink rate in response to A77636 at any of the three doses was not correlated with D₁-like receptor availability in any of the striatal regions examined (all p 's>0.12).

To determine if the relationship between D₂-like receptor availability and change in average eye blink rate in response to PHNO was driven by an *increase* in eye blink rate, we examined the relationship between D₂-like receptor availability and the change in maximal/minimal eye blink rate following administration of PHNO. Consistent with this hypothesis, the change in maximal, and not minimal, eye blink rate following administration of 0.005 and 0.05 mg/kg dose of PHNO was positively correlated with D₂-like receptor availability (0.005 mg/kg: caudate nucleus $r_{10}=0.64$, $p=0.04$; putamen $r_{10}=0.61$, $p=0.06$; ventral striatum $r_{10}=0.91$, $p<0.001$; 0.05 mg/kg: caudate nucleus $r_{10}=0.65$, $p=0.04$) (Figure 3A-F), indicating that the differences in D₂-like receptor availability were reflective of functionally relevant receptor pools. The change in maximal or minimal eye blink rate following administration of A77636 did not correlate with D₁-like receptor availability in any of the regions examined (all p 's>0.08).

As described above, average eye blink rate was extremely stable before the pharmacological portion of the experiment. To confirm that this stability was not influenced across the 10-week dopamine agonist challenge (i.e., sensitization of dopamine receptors produced by multiple administrations of dopamine receptor agonists), reliability of average eye blink rate in the baseline recording sessions of the four saline sessions was examined. Eye blink rate was extremely reliable across the four saline administration sessions (Cronbach's alpha: 0.96), suggesting that the multiple administrations of dopamine agonists did not alter spontaneous eye blink rates.

In vitro neurochemical measures of dopamine: The pharmacological data described above indicated that the differences in D₂-like receptor availability measurements reflected differences in functionally relevant receptor pools. Next, we sought to test the possibility that differences in D₂-like receptor availability measured with PET were influenced by differences in endogenous levels of dopamine that could compete with the radiotracer. Levels of dopamine measured in striatal homogenates were not significantly correlated with D₁-like or D₂-like receptor availability within the caudate nucleus (all p 's > 0.15; Figure 4A) or the putamen (all p 's > 0.32; Figure 4B); a trend-level positive correlation was detected between levels of dopamine and D₂-like receptor availability in the ventral striatum ($r=0.60$; $p=0.07$; Figure 4C), but not for D₁-like receptor availability ($r=0.11$; $p=0.76$). However, the ratio of homovanillic acid to dopamine levels, which is thought to reflect dopamine turnover more directly (Sharp et al., 1986), was not correlated with D₂-like or D₁-like receptor availability in any of the regions examined (all p 's > 0.21).

Levels of dopamine in the caudate nucleus have been reported to be correlated with spontaneous eye blink rate in monkeys exposed to dopaminergic-toxic drug MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Taylor et al., 1999). To determine if individual differences in levels of dopamine could explain the differences in spontaneous eye blink rate detected in this sample of

monkeys, correlations were conducted between dopamine levels and average eye blink rate for the 30 min prior to administration of saline, as these were collected closest in time to tissue collection. Average eye blink was not statistically related to levels of dopamine in any of the striatal regions examined (all $p's > 0.21$).

[³H]spiperone assessment of D₂-like receptor density: As mentioned above, technical challenges and time restrictions prevented the assessment of [³H]spiperone binding in the putamen or nucleus accumbens; however, [³H]spiperone binding was assessed in tissue collected from the caudate nucleus for nine of the ten subjects. Maximal number of [³H]spiperone binding sites (B_{max}) was, on average, 197 +/- 45.85 fmol/mg of tissue, while the mean value of the equilibrium dissociation constant, K_d , was 0.10 nM, consistent with the results of previous studies (Alexander et al., 1991). We then tested whether B_{max} for [³H]spiperone or K_d were correlated with the PET measurements of D₂-like receptor availability in the caudate nucleus. Remarkably, BP_{ND} in the caudate nucleus was positively correlated with B_{max} for [³H]spiperone ($r_9=0.71$; $p=0.03$; Figure 5A), but not with the K_d ($r_9=0.15$; $p=0.71$; Figure 5B), suggesting that the variation in D₂-like receptor availability is due to differences in D₂-like receptor density, rather than affinity of the receptor.

To determine if spontaneous eye blink rate was correlated with density of D₂-like receptors, we examined the relationship between B_{max} for [³H]spiperone and average eye blink rate for the 30 min prior to administration of saline, as these were collected closest in time to the brain tissue collection. B_{max} for [³H]spiperone was positively correlated with average eye blink rate ($r_9=0.77$; $p=0.01$; Figure 5C), but K_d was not ($r=0.47$; $p=0.20$).

Behavioral relevance of differences in eye blink rate: To provide evidence for the behavioral relevance of differences in eye blink rate, as a proxy of D₂-like receptor function, the relationship between eye blink rate and the behavioral performance of monkeys in the reversal phase was examined. Spontaneous eye

blink rate was not correlated with the number of trials required to reach criterion in the reversal phase ($r_9=-0.40$; $p=0.29$; Figure 6A) but was associated with the probability of monkeys persisting with the correct response following positive feedback ($r_9=0.68$; $p=0.04$; Figure 6B). Spontaneous eye blink rate was not correlated with the sensitivity of individuals to negative feedback (all $p's > 0.84$).

Discussion

By combining neuroimaging measurements with behavioral and neurochemical assessments of the dopamine system, the current study accomplishes two goals. First, it generates direct evidence that the differences in brain dopamine D₂ receptor availability measured with PET and related to liability for addictions is linked to altered dopamine D₂-like receptor density. Second, it characterizes a minimally invasive phenotype (eye blink rate) that both predicts brain dopamine D₂-like receptor availability and that reveals the functional relevance of the dopamine receptors measured with PET.

The present data add to the growing literature showing that striatal dopamine D₂-like receptor availability is associated with impulsivity, inhibitory control abilities and substance abuse liability (Nader et al., 2006; Dalley et al., 2007; Lee et al., 2009; Buckholtz et al., 2010a; Ghahremani et al., 2012). Our studies add substantially to this literature by providing the first direct evidence that behaviorally significant variation in D₂-like receptor availability reflects differences in the density of functionally active D₂-like receptors. D₂-like receptor availability assessed with PET was positively correlated with spontaneous and D₂-like receptor agonist-evoked changes in eye blink rate, as well as with [³H]spiperone binding to striatal homogenates, assessed *in vitro*. Together, these results provide the necessary framework needed for a more complete interpretation of PET measurements of D₂-like receptors and their relationship to susceptibility for addictive behaviors.

D2-like receptor modulation of inhibitory control and feedback sensitivity

Previous results, collected in a different cohort of monkeys, indicated that D₂-like receptor availability in the dorsal striatum, but not the ventral striatum, was related to reversal learning performance and behavioral sensitivity to positive feedback during learning (Groman et al., 2011); however, the current study found that D₂-like receptor availability in the ventral, but not dorsal, striatum was correlated with reversal learning performance and positive-feedback sensitivity. Although

these studies, together, provide supporting evidence that the D₂-like receptor is involved in the behavioral flexibility in response to positive feedback, they differ in concluding which striatal nuclei govern these processes. One possibility is that multiple striatal regions influence this process and that statistical threshold and/or power issues influence which brain region is detected in different cohorts of subjects. Another possibility is that the neural circuitry underlying learning during reversal conditions changes as a function of experience with the task. Several studies indicate that the neural mechanisms necessary for behavioral flexibility during reversal learning change as a function of experience with reversal (Boulougouris and Robbins, 2009; Izquierdo and Jentsch, 2012), and the monkeys used in the current study had considerably less experience with the reversal learning paradigm than did the subjects in our previous study (Groman et al., 2011).

The mechanism by which D₂-like receptors influence the ability to modify behavior adaptively through the effective use of positive feedback is unknown. The dopamine system has been established as being critical to the learning of stimulus-reward associations: changes in the firing of midbrain dopaminergic neurons that occur when expectancies are violated have been proposed to encode the prediction errors that drive learning (Fiorillo et al., 2003). Computational models of the basal ganglia have suggested that these slight changes in dopaminergic tone by positive and negative outcomes exert differential effects on D₁-like and D₂-like expressing medium spiny neurons of the striatum (Frank et al., 2004). One such model hypothesizes that phasic increases in dopamine act through D₁-expressing medium spiny neurons to promote learning from positive feedback, while dips in dopamine tone that occur following negative outcomes are detected by D₂-expressing medium spiny neurons promoting learning from negative feedback (Frank and O'Reilly, 2006). Collectively, our studies have not provided evidence supporting these dissociable contributions of D₁-like and D₂-like receptors to learning. Instead, the results of the current study suggest that dopamine acting through the D₂-like receptors largely governs the ability of subjects to utilize positive outcomes to guide behavior effectively; the mechanisms

underlying the sensitivity of individuals to negative feedback is unexplained by our data, but new data indicate a potential role for the amygdala (Rudebeck and Murray, 2008; Izquierdo et al., 2013).

Differences in D₂-like receptor availability reflect differences in the density of functional D₂-like receptors

Low D₂-like receptor availability detected with PET imaging in substance dependent individuals and animals chronically exposed to drugs (Volkow et al., 1993; Volkow et al., 2001b; Nader et al., 2006; Lee et al., 2009; Groman et al., 2012) have been hypothesized to reflect changes in the density of D₂-like receptors. However, until now, the comparison between *in vivo* and *in vitro* assessments of D₂-like receptors, in this context, had not been conducted. We provide evidence that individual variability in striatal D₂-like receptor availability is correlated with the density of striatal D₂-like receptors, assessed using [³H]spiperone binding. Notably, this correlation was detected despite separated period of approximately 3 years between the collection of the *in vivo* and *in vitro* data, demonstrating a remarkable stability of D₂-like receptor density. These differences are apparently not determined by tissue dopamine levels or by differences in affinity of the receptor for the ligand(s). Further, the correlations between D₂-like receptor availability and pharmacological-induced changes in eye blink rate provide evidence that in the individual differences in D₂-like receptor availability reflect receptors coupled to their intracellular signaling pathways / biologically meaningful receptor pools.

The precise etiology for the individual variation in D₂-like receptor density detected in the current study is not known, but may be due to genetic and/or early environmental factors that influence the expression of D₂-like receptors. For example, humans that carry the Taq1A polymorphism, located in the ankyrin repeat and kinase domain containing 1 gene that influences brain dopamine synthesis (Laakso et al., 2005), have lower striatal D₂-like receptor availability than non-carriers (Pohjalainen et al., 1998). Alternatively, alterations of environmental features, such as housing structure and social dominance position, can influence D₂-like receptor availability measurements (Morgan et al., 2002).

Because D₂-like receptor availability and [³H]spiperone binding, measured 3 years apart, were correlated, the differences in D₂-like receptor availability detected in this sample of vervet monkeys is most likely due to the influence of genes that alter the activity of the dopamine system, as well as by a careful experimental control of environmental factors. Although the polymorphism homologous to the Taq1A polymorphism in humans has yet to be identified in vervet monkeys, a quantitative trait locus for variation in levels of homovanillic acid in CSF has been revealed (Freimer et al., 2007) and a mechanism like this could also indirectly affect D₂-like receptor density through altering synaptic dopamine utilization.

Spontaneous eye blink rate as a behavioral correlate of D2-like receptors

Spontaneous eye blink rate is altered in disorders in which central dopamine function is compromised. Spontaneous eye blink rate is lower in individuals with Parkinson's disorder (Hall, 1945) and is greater in those diagnosed with schizophrenia (Karson et al., 1990) in comparison to otherwise healthy human subjects. The influences of dopamine on spontaneous eye blink rate may occur within the striatum given that levels of dopamine within the caudate nucleus correlate with spontaneous eye blink rate in a primate model of Parkinson's disease (Taylor et al., 1999), and drugs that act on dopamine receptors, whose density is greatest in the striatum, alter eye blink rate (Elsworth et al., 1991; Kleven and Koek, 1996). The current study provides additional support for the relationship between the dopamine system and spontaneous eye blink rate, demonstrating that naturally occurring variation in striatal D₂-like receptor availability is positively correlated with spontaneous eye blink rate. Our data therefore provide initial support for the notion that eye blink rate is a minimally invasive measure that captures a substantial proportion of the variance in dopamine receptor availability measured with PET.

Unlike eye-blink conditioning, the circuitry underlying spontaneous eye blink is not well understood; most studies have been directed at investigating the circuitry underlying reflex blinks, or

stimulus-evoked blinks (i.e. airpuff or touch to the cornea). Nevertheless, it has been postulated that the same circuit may also govern spontaneous, and sensory-evoked, eye blinks (Kaminer et al., 2011). In the reflex blink circuit, multimodal sensorimotor information is integrated within the intermediate and deep layers of superior colliculus, via projections from cortical regions (i.e. frontal eye fields and prefrontal regions), the substantia nigra pars reticulata and the visual cortex. The superior colliculus sends excitatory projections to the nucleus raphe magnus (Basso et al., 1996; Gnadt et al., 1997), which exerts inhibitory control over the spinal nucleus of the trigeminal nerve (Basso and Evinger, 1996). The spinal nucleus of the trigeminal nerve then sends excitatory projections to the orbicularis oculi motoneurons in the facial nucleus to innervate the orbicularis oculi muscles that close the eye lid (van Ham and Yeo, 1996).

The influence of the dopamine system on the reflex blink circuit is thought to occur via inhibitory projections of the substantia nigra pars reticulata onto neurons within the superior colliculus (Basso et al., 1996). Because medium spiny neurons of the striatum receive input and project back to the substantia nigra (Haber et al., 2000), dopamine receptors expressed on striatal neurons can directly modify activity of the substantia nigra (Akkal et al., 1996) and thereby influence reflex blinks by altering the activity of the superior colliculus, and the nuclei downstream that innervate the muscles controlling eye blinks. In line with this is evidence that dopamine agonists increase blink rate (Elsworth et al., 1991) while dopamine receptor antagonists decrease blink rate (Kleven and Koek, 1996). However, the results of the current study suggest that there is a selective relationship between spontaneous eye blink rates and D₂-like receptor density, which cannot be accounted for based on these hypothesized influences of dopamine within the reflex blink circuitry. This remains an open research question and will require further studies to determine the precise way in which D₂-like receptor signaling influences spontaneous eye blink rate.

Implications for substance use disorders

Improving D₂-like receptor function has been proposed as a treatment strategy for substance use disorders for decades (Dackis et al., 1985), and clinical trials using D₂-like receptor agonists have been conducted (Gorelick and Wilkins, 2006). The results of these trials have generally been discouraging (Soares et al., 2001). Although D₂-like receptor agonists increase D₂-mediated signaling, albeit in a physiologically irrelevant manner, it is possible that treatment strategies that rescue the dampened D₂-like receptor density, rather than simply enhancing the activity of the receptors that are expressed, would be more effective in the treatment of substance dependence. In line with this hypothesis is evidence that viral-mediated overexpression of D₂-like receptors in striatal regions reduces self-administration of cocaine and alcohol in rats (Thanos et al., 2004; Thanos et al., 2008). However, there are currently no techniques for increasing D₂-like receptor density in humans. Recent work has indicated that exercise may increase striatal D₂-like receptor availability in methamphetamine-dependent individuals (Robertson et al., *unpublished*). Although the impact of the D₂-like receptor increase on measures of sobriety remains to be determined, exercise may provide a naturalistic tool for improving D₂-like receptor function in substance-dependent populations.

Figures

Figure 4.1: The relationship between D₂-like receptor availability in the caudate nucleus (dark circles), putamen (gray circles) and ventral striatum (open circles) and the average number trials required to reach criterion in the reversal phase (A). Panels B and C shows the relationship between D₂-like receptor availability in the ventral striatum and the probability of individual subjects persisting with a correct response following positive feedback (B) and the probability of individual subjects making a perseverative response following positive feedback (C).

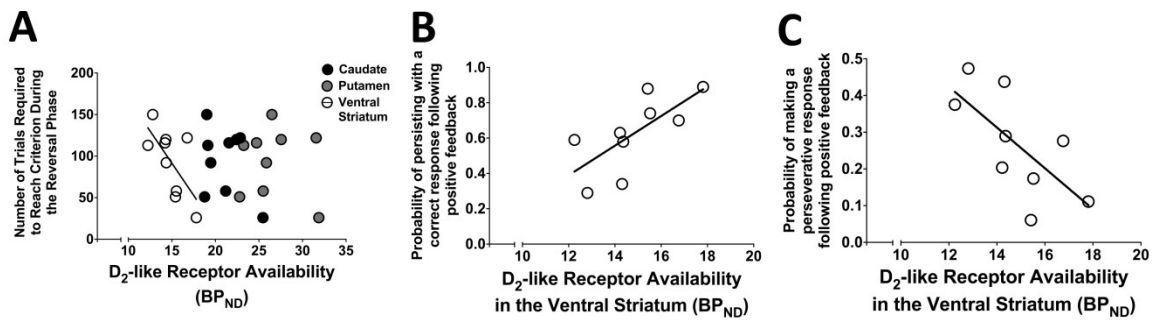


Figure 4.2: The reliability and stability of spontaneous eye blink rates. Average eye blink rate, in 5 min bins, across the first 60 min assessment (SEBR Session 1; A) and the correlation between the two 60 min spontaneous eye blink rate assessments

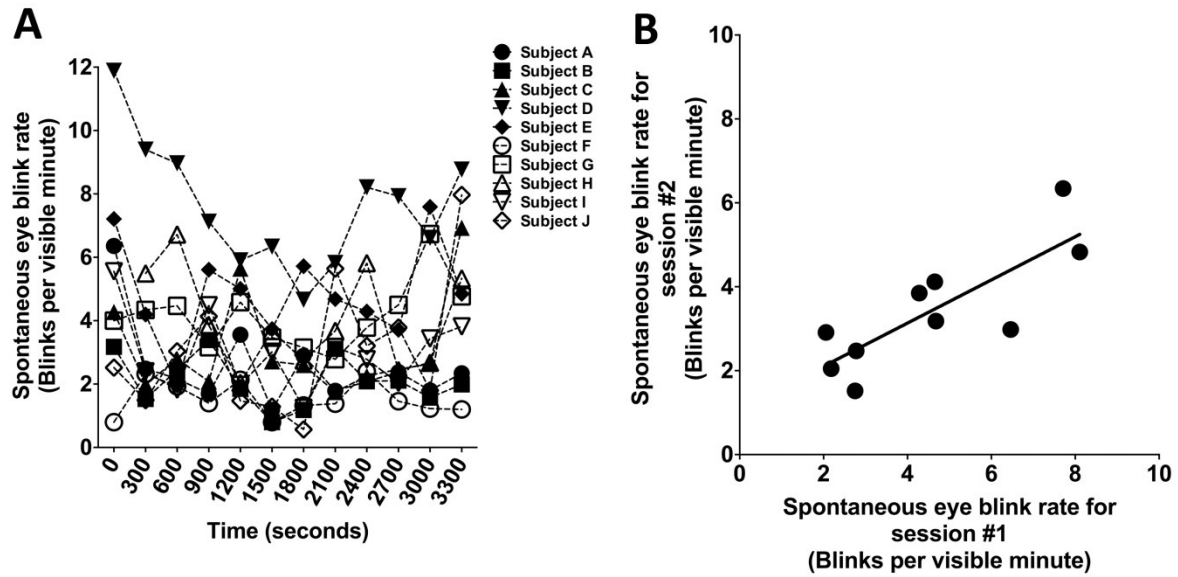


Figure 4.3: The relationship between dopamine receptor availability (D_1 -like receptors, open circles; D_2 -like receptors, closed circles) and spontaneous eye blink rate in the caudate nucleus (A), the putamen (B) and the ventral striatum (C). Statistical maps (p values) from the voxel-wise regression of D_2 -like receptor availability and spontaneous eye blink rate from a transverse (D), coronal (E) and sagittal (F) section. TFCE images were overlaid on the MR template with results thresholded at TFCE-corrected $p < 0.10$.

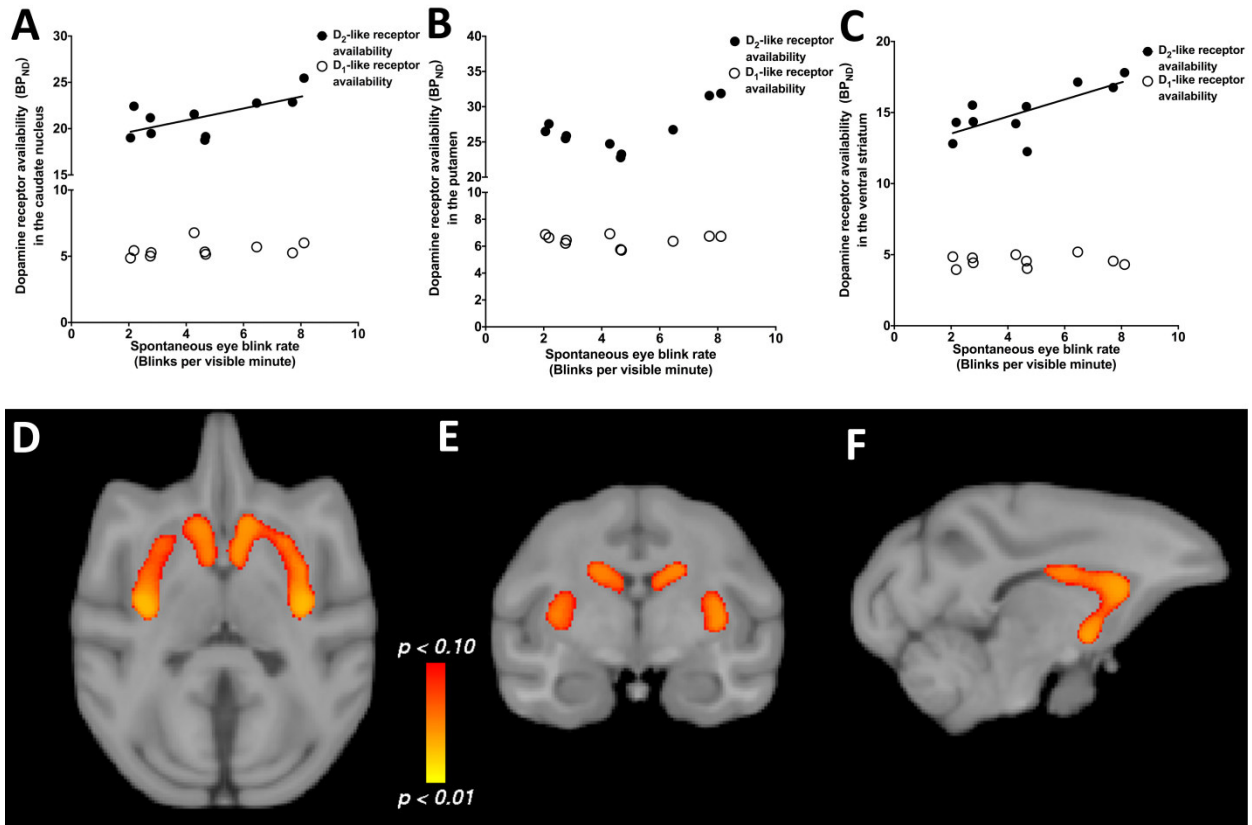


Figure 4.4: The relationship between striatal D₂-like receptor availability (caudate nucleus, closed circles; putamen, gray circles; ventral striatum, open circles) and the change in maximal eye blink rate in response to the 0.005 mg/kg (A), 0.05 mg/kg (B) and 0.5 mg/kg (C) dose of the D₂-like receptor agonist, PHNO. Statistical maps (p values) from the voxel-wise regression of change in maximal eye blink rate following administration of PHNO on D₂-like receptor availability for the 0.005 mg/kg (D) and 0.05 mg/kg (E) dose of PHNO. The overlap of these two statistical maps is presented in Panel F. TFCE images were overlaid on a MR template with results thresholded TFCE-corrected $p < 0.05$.

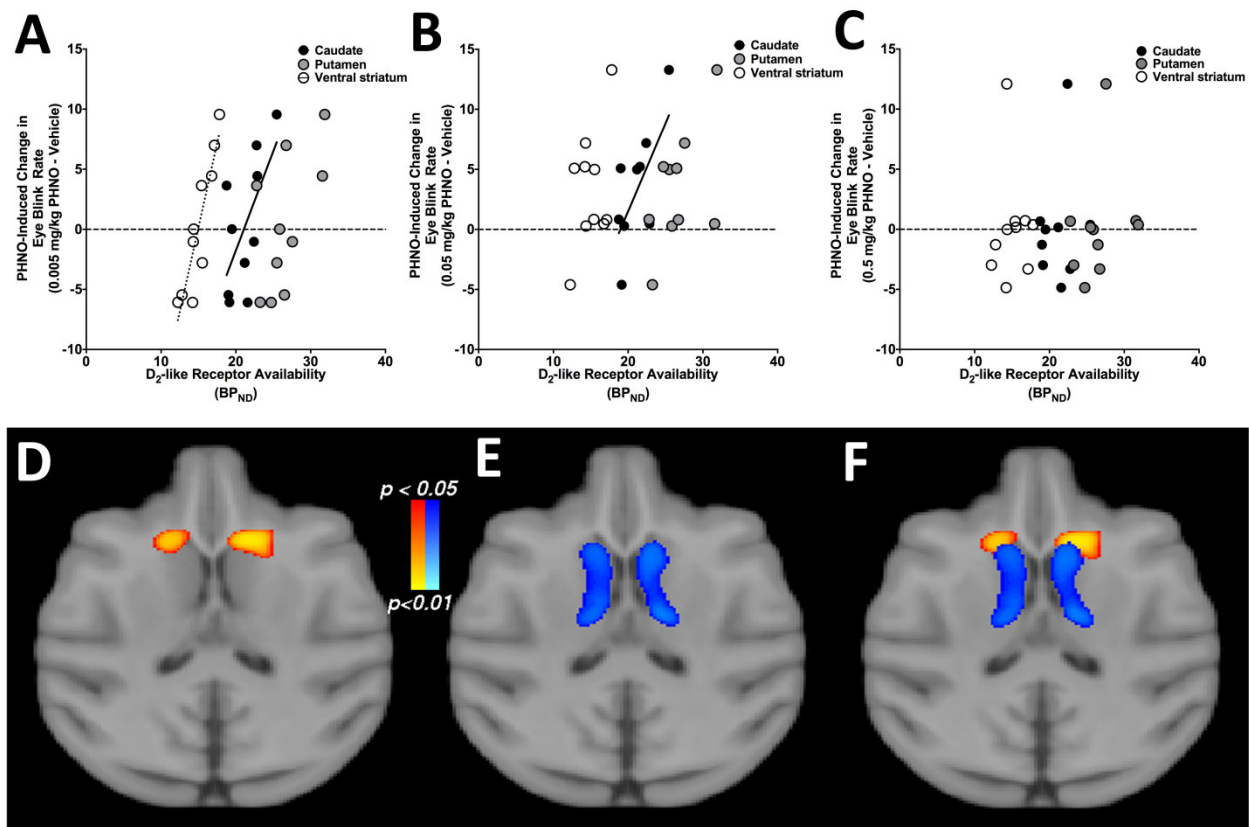


Figure 4.5: Scatter-plots of the relationships between levels of dopamine and D₂-like receptor availability in the caudate nucleus (A), the putamen (B) and ventral striatum (C).

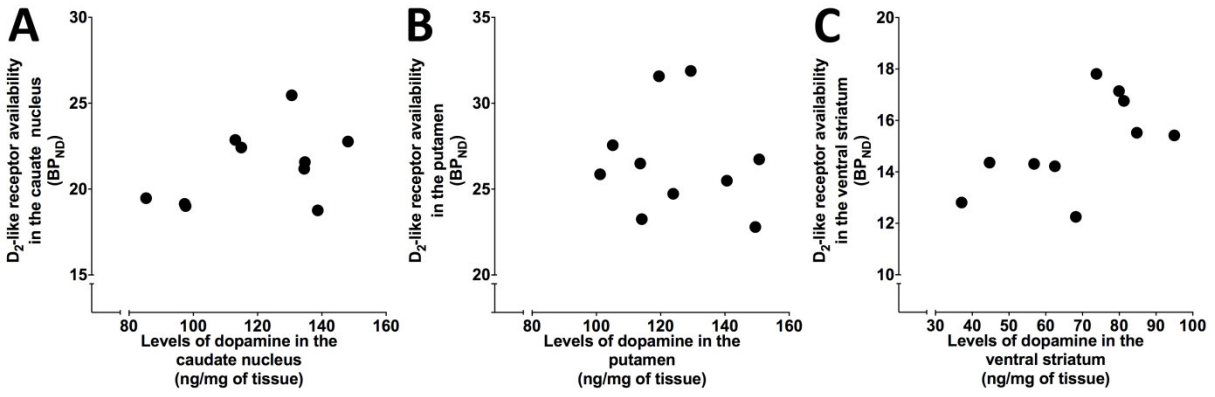


Figure 4.6: The relationship between D₂-like receptor availability (BP_{ND}) in the caudate nucleus and maximal number of [³H]spiperone binding sites (A) and the equilibrium constant of [³H]spiperone (B) in the caudate nucleus. The relationship between the maximal [³H]spiperone binding sites and spontaneous eye blink rate is presented in panel C.

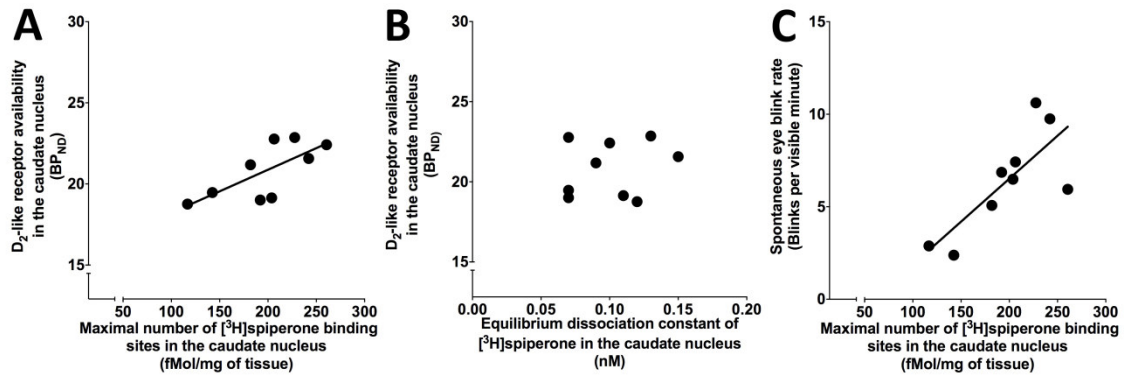
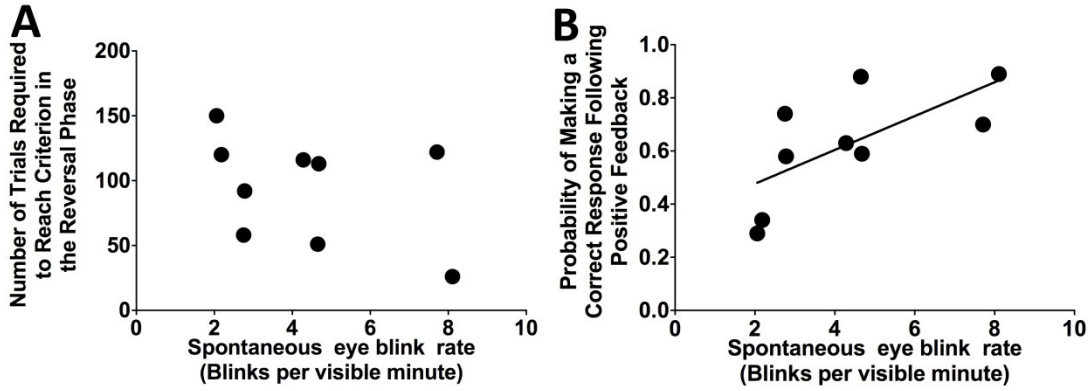


Figure 4.7: The relationship between spontaneous eye blink rate and number of trials required to reach criterion in the reversal phase (A) and the probability of monkeys persisting with a correct response following positive feedback (B).



Chapter 5

Spontaneous eye blink rate and D₂-like receptor density is greater in rats following chronic haloperidol exposure

Summary

Despite the importance of relationships between in vivo measures of brain dopaminergic function and psychopathology, the techniques currently available for assessing these biochemical processes non-invasively with positron emission tomography (PET), are technically challenging, costly and laborious, limiting their utility in large samples or in smaller or less well-developed research institutions. Our work in non-human primates (Chapter 4) has revealed that spontaneous eye blink rate is remarkably trait-like and is positively correlated with D₂-like, but not D₁-like, receptor availability in the striatum, suggesting that eye blink rate could serve as a non-invasive proxy for D₂-like receptor function. To provide further support for this possibility and to examine whether such a relationship holds in more commonly used laboratory model systems, spontaneous eye blink rate was assessed in 17 (9 M/ 8 F) Long-Evans rats before and after daily treatment with the dopamine D₂ receptor antagonist haloperidol (or vehicle) for 30 days. After the behavioral phenotypes were collected, *individual estimates of D₂-like receptors* were made in striatal tissue homogenates, using [³H]spiperone. Spontaneous eye blink rate increased in rats that received daily injections of haloperidol, but not those that received vehicle. The maximal number of binding sites of [³H]spiperone was greater in haloperidol-exposed rats compared to vehicle-exposed rats, with no group differences being detected in the equilibrium dissociation constant. Maximal number of binding sites of [³H]spiperone was correlated with spontaneous eye blink rate when sex was controlled for, indicating that sexually-biasing factors may influence this relationship. These data support the notion that EBR may be an effective tool for longitudinally monitoring dopamine D₂ receptor number and function in rodent models.

Introduction

The dopamine D₂-like receptor has been identified as a crucial indicator in the pathophysiology of a number of mental disorders, such as schizophrenia, addiction, Tourette syndrome, and bulimia nervosa (Wong et al., 1986; Volkow et al., 1993; Wong et al., 1997; Broft et al., 2012). Neuroimaging studies, indexing D₂-like receptor availability, have indicated that the degree of receptor dysfunction covaries with pathological dimensions of these disorders, such as symptom severity and cognitive dysfunction (Wong et al., 1997). Although the directionality of D₂-like dysfunction differs between these disorders (lower D₂-like receptor availability in substance dependent individuals and greater D₂-like receptor availability in schizophrenia), improving D₂-like receptor signaling has been proposed as a primary treatment strategy for many of these disorders (Kosten et al., 2002; Steeves and Fox, 2008; Costentin, 2009).

In humans, D₂-like receptors are assessed using neuroimaging techniques, such as positron emission tomography, which are technically challenging, costly, and labor-intensive, limiting their utility in large samples or in research institutions that lack the necessary equipment to conduct such sophisticated studies. Moreover, the potential health-risks associated with exposure to ionizing radiation generally limit the use of these techniques to adults, which in the case of many psychopathologies is beyond the period where symptoms first appear (Crews et al., 2007; Paus et al., 2008), and past the developmentally-sensitive time period where intervention strategies are most effective (Larsen et al., 2001; Webster-Stratton and Taylor, 2001; Harrigan et al., 2003).

Although PET has been used in non-human primates and rodents to measure D₂-like receptors, providing supporting evidence for the alterations detected in humans (Groman and Jentsch, 2013a), the same factors that limit the use of PET in humans deter many researchers from using PET in animal models of psychopathology. Instead, post-mortem techniques, such as a radioligand binding assays, are used to measure D₂-like receptors in animals. These methods do provide high biological resolution but

do not permit repeated measurements to be collected in the same subject. Although PET and radioligand binding assays are based on the same principles, the measurements and their interpretation can be very different, resulting in a translational disconnect of results across species.

In chapter 4, we report that spontaneous eye blink rate in non-human primates is highly trait-like and related to D₂-like, but not D₁-like, receptor availability in the striatum. We proposed that spontaneous eye blink rate may represent a non-invasive, easily-quantifiable proxy of D₂-like receptors which could be used repeatedly, in longitudinally designs, to index D₂-like receptor function in large samples of individuals and identical in humans and animals. However, the applicability of the observed relationship between spontaneous eye blink rate and D₂-like receptors in other species is unknown.

In order to provide additional evidence supporting the relationship between D₂-like receptor density and eye blink rate, as well as provide cross-species evidence for this relationship, we measured eye blink rate in rodents before and after chronic administration of haloperidol (or vehicle), which produces a robust increase in striatal D₂-like receptor density (Fleminger et al., 1983; MacKenzie and Zigmond, 1985). D₂-like receptor density was assessed *in vitro* in the dorsolateral striatum using [³H]spiperone. Based on our previous results, we hypothesized that chronic haloperidol exposure would increase eye blink rate, as well as striatal D₂-like receptor density, and that eye blink rate would be related to D₂-like receptor density.

Methods

Subjects: Seventeen Long-Evans rats (Harlan, 9 M/8 F) between 6-10 months of age were included in the study. Food and water were available *ad libitum* throughout the experiment, except during the 40-min video recording sessions. All subjects were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85–23, revised 2010. Research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Drugs: Haloperidol was purchased from Sigma-Aldrich (St Louis MO) and was dissolved in a vehicle containing 5% DMSO and 5% TWEEN 80, in sterile water. Drug (and vehicle) solutions were prepared fresh daily and were administered for 30 consecutive days at 0.1 ml/kg via the intraperitoneal route, between the hours of 700 and 900.

Eye blink recording: Rats were recorded individually using two high-definition digital video cameras (Boggie HD, Sony). Rats were placed in a custom-made transparent Plexiglas runway (47 x 14 x 22 cm), in which the width was adjustable and was changed to encourage the rats to always face one of the two cameras. The two video cameras were positioned on opposite sides of each other, recording subjects through the transparent ends of the runway.

Prior to the measurement of eye blink rate, rats were placed in the chamber for 40 min on two consecutive days, in order to reduce the anxiety and increased locomotion that can occur in response to novel environments. Eye blinks in rats were then video-recorded for 40 min on two consecutive days; a week later, daily injections with haloperidol or vehicle commenced. A 2 d period was allowed to elapse between the 30 days of treatment and EBR assessments. After this period, rats were re-habituated to the chamber for 40 min on one day and eye blinks recorded for 40 min on the following 2 days.

Preparation of membrane homogenates: Within 2 h of the last video recording session, rats were sacrificed by decapitation, and brain regions were isolated by free hand dissection on a cold platform (Raymond Lamb). Tissue was extracted from the dorsolateral striatum, the dorsomedial striatum and nucleus accumbens, weighed and stored in a -80°C freezer until processed. Tissue was homogenized in 20 volumes of assay buffer (50 mM Tris HCl, 50 mM Tris Base, 5 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 , pH to 7.4 at 23°C), using a tissue sonicator (Sonic Dismembrator Model 100, Fisher Scientific). Tissue homogenate was centrifuged twice at 15,000 RCF for 15 min, resuspending the pellet each time in 20 volumes of assay buffer. The final tissue pellet was suspended in assay buffer to yield a final tissue concentration of 3 mg original wet tissue weight (o.w.w.) per ml of buffer.

[^3H]Spiperone binding assays: Binding assays were performed in duplicates in disposable polystyrene tubes in a final volume of 0.5 ml using procedures previously described (Levant, 2007). A ten-point saturation curve was conducted, with the final concentration of [^3H]spiperone at approximately 5 nM. Binding was initiated by the addition of membrane homogenate (3 mg/ml o.w.w.) to the incubation containing [^3H]spiperone. Nonspecific binding was defined in the presence of 5 μM (+)-butaclamol. Samples were incubated for 90 min at 27°C , and the reaction was terminated by separation of the free from the bound radioligand using rapid vacuum filtration over Whatman GF/B filters (pre-soaked in 0.5% PEI at room temperature for 90 min) using a Brandel Cell Harvester. Filters were washed 3 times with 2 ml of ice-cold wash buffer (50 mM Tris-HCl and Tris-Base, pH 7.4 at 27°C) and were allowed to dry before being placed in 2 ml of liquid scintillation fluid (Ultima Gold, Perkin Elmer). Filters were soaked for no less than 6 hours, but no more than 48 hrs, in the scintillation fluid before being counted in a liquid scintillation counter (Beckman LS 6500, Beckman Coulter). Specific binding of [^3H]spiperone was determined by subtracting nonspecific binding from total binding and was expressed as femtomoles per milligrams of protein. Protein concentration was determined using procedures described by Lowry (Lowry et al., 1951).

Data processing:

Determining eye blink rate: Videos were viewed by experimentally-blind scorers, who marked the occurrence of a spontaneous eye blink (defined as a full opposition of the eye lid that was not elicited by the subject, such as those that occur during grooming) and changes in visibility of the rat using an in-house program. Only the final 20 min of the 40 min video recording sessions were scored, with the video being played back at half speed in order to improve accuracy of the ratings. Spontaneous eye blink rate (blinks/min) was determined by dividing the total number of blinks that occurred within a session by the total time each subject was visible. Eye blink rate for the two days prior to initiating the drug regimen and the two days after the dosing regimen were averaged (referred to as the “baseline eye blink rate” and “post drug-regimen eye blink rate”, respectively).

Determining D₂-like receptor density and affinity: Saturation curves, representing the relationship between specific binding of [³H]spiperone and radioligand concentration, were created for each subject and analyzed using nonlinear regression in GraphPad (Prism, version 5). The maximal number of [³H]spiperone binding sites (B_{max}) and the equilibrium dissociation constant (K_d) were determined from the fit of these nonlinear curves.

Statistics: All statistical analyses were completed in SPSS (v 15.0). The effect of the 30-day dosing regimen on eye blink rate was examined using paired samples t-tests between the vehicle and haloperidol groups. D₂-like receptor density (B_{max}) and D₂-like receptor affinity (K_d) were compared between the vehicle and haloperidol groups using independent samples t-tests. Multiple linear regression was used to examine the relationship between eye blink rate and measures of [³H]spiperone binding, while controlling for sex.

Results

Characteristics of eye blink rate in rodents: Average eye blink rate prior to initiating the drug regimen was 2.57 +/- 0.29 (mean +/- SEM) blinks per min. Eye blink rates in the two consecutive recording sessions completed prior to the drug regimen were constitutively correlated ($R(16)=0.55$; $p=0.03$), indicating that eye blink rate was similar in these subjects across the two days.

Haloperidol exposure increases eye blink rate: Eye blink rate did not differ between groups prior to the 30-d administration of haloperidol or vehicle ($t(15)=0.11$; $p=0.91$); however, eye blink rate was significantly different between the two groups following the dosing regimen ($t(15)=3.58$; $p=0.003$). Paired t-tests indicated that exposure to haloperidol significantly increased eye blink rate from baseline ($t(8)=2.56$; $p=0.03$; baseline eye blink rate: 2.61 +/- 0.37; post-drug eye blink rate: 3.75 +/- 0.15), while administration of vehicle did not significantly alter eye blink rate ($t(7)=-0.70$; $p=0.51$; baseline eye blink rate: 2.54 +/- 0.49; post-drug eye blink rate: 2.84 +/- 0.20) (Figure 2).

Effects of chronic haloperidol exposure on striatal D₂-like receptor density: Consistent with previous studies, [³H]spiperone binding was significantly greater in rats chronically dosed with haloperidol compared to those exposed to vehicle ($t(15)=3.65$; $p=0.002$; Figure 3A). The difference in [³H]spiperone binding reflected an effect on B_{max} , as K_d was not statistically different between the groups ($t(15)=0.87$; $p=0.40$; Figure 3B).

Correlation between eye blink rate and D₂-like receptor density: The relationship between average eye blink rate following the drug regimen and D₂-like receptor density was examined with Pearson's product-moment correlation coefficient. D₂-like receptor density was not correlated with the average eye blink rate following the drug regimen ($R(17)=0.30$; $p=0.24$). The relationship was also non-significant when the two groups were evaluated separately (haloperidol: $R(9)=0.064$, $p=0.87$; vehicle: $R(8)=-0.669$; $p=0.07$). To determine if sex was influencing the relationship between [³H]spiperone binding and

average eye blink rate following the drug regimen, multiple linear regression was used to regress sex (dummy coded) and [³H]spiperone binding against eye blink rate. The model including sex and [³H]spiperone binding explained 37% of the variance in average eye blink rate ($F_{2,14}=4.03$; $p=0.04$). Consistent with our previous findings, [³H]spiperone binding was positively associated with eye blink rate ($\beta=0.603$; $t=2.45$; $p=0.03$); however, sex was negatively correlated with eye blink rate ($\beta=-0.607$; $t=-2.47$; $p=0.03$) (Figure 4).

Discussion

The results of this study indicate that eye blink rate is altered following chronic administration of haloperidol using a dosing regimen previously shown to increase D₂-like receptor density; exposure to a 30-d regimen of haloperidol increased eye blink rate and D₂-like receptor density in the dorsolateral striatum, in comparison to exposure to vehicle, providing further evidence supporting the relationship between D₂-like receptors and eye blink rate. Importantly, these data extend our previous findings in non-human primates to rodents, providing evidence for the application of eye blink rate as a proxy for D₂-like receptors across species.

Chronic haloperidol exposure increases eye blink rate

Chronic blockade of the D₂-like receptor, using a protocol known to increase striatal D₂-like receptor density as demonstrated here, increases eye blink rates in a rodent model. These results, in combination with our previous findings, support the hypothesis that eye blink rate may serve as a non-invasive behavioral proxy for D₂-like receptors. Although we believe that the changes in eye blink rate that occurred following the haloperidol exposure are due to changes in D₂-like receptor density, it is also possible that other dopamine-dependent mechanisms contributed to this behavioral change, as chronic administration of haloperidol alters other aspects of the dopamine system, such as striatal levels of dopamine and dopamine metabolites, which are reduced (Moore et al., 1998). Despite these gross alterations in dopamine tone, the effect of haloperidol on dopamine receptors appears to be selective to the D₂-like receptor: rats chronically exposed to haloperidol have greater [³H]spiperone binding than control rats, but no changes in binding of the D₁-like receptor antagonist [³H]SCH 23390 (Savasta et al., 1988). Although [³H]SCH 23390 binding was not assessed in the current study, the lack of an effect of haloperidol on D₁-like receptors reported by previous studies (MacKenzie and Zigmond, 1985; Savasta et al., 1988; Sanci et al., 2002) provides compelling evidence that the change in eye blink rate detected here is most likely not due to changes in D₁-like receptors, but changes in D₂-like receptor density. It is

also possible that changes in dopamine tone account for the change in eye blink rate; however, if the change in eye blink rate were driven by reductions in dopamine levels or utilization, then eye blink rate would have decreased, rather than increase, following the drug regimen. Therefore, the haloperidol-induced increases in eye blink rate are most likely due to upregulation of D₂-like receptors.

Although the current study did not detect a correlation between D₂-like receptor density and eye blink rate, it is possible that utilization of female rats in the current study introduced variables that were not explicitly controlled for, such as different phase of the estrous cycle amongst the female rats. There is evidence that dopamine-elicited behaviors vary across the different phases of the estrous cycle (Becker and Cha, 1989) and such differences may have impeded our ability to detect the correlation between D₂-like receptor density and eye blink rate. Our results are in line with this view, in that when sex was included as a co-variate in the regression model, [³H]spiperone binding was positively related to eye blink rate. However, as we did not account for differences in estrous phase among the female rats, it is unclear which of the sex-biasing biological factors contributes to this effect the most. Additional studies accounting for these physiological parameters may provide insight into the nature of the sex difference detected in the current study.

Potential circuitry underlying spontaneous eye blink rate

Unlike eye-blink conditioning, the circuitry underlying spontaneous eye blink is not well understood and most studies have been directed at investigating the circuitry underlying reflex blinks, or stimulus-evoked blinks (i.e. airpuff or touch to the cornea). However, it has been postulated that this same circuit may also govern spontaneous eye blinks (Kaminer et al., 2011). In the reflex blink circuit, multimodal sensorimotor information is integrated within the intermediate and deep layers of superior colliculus via projections from cortical regions (i.e., frontal eye fields and prefrontal regions), the substantia nigra pars reticulata and the visual cortex. The superior colliculus sends excitatory projections

to the nucleus raphe magnus (Basso et al., 1996; Gnadt et al., 1997), which exerts inhibitory control over the spinal nucleus of the trigeminal nerve (Basso and Evinger, 1996). The spinal nucleus of the trigeminal nerve then sends excitatory projections to the orbicularis oculi motoneurons in the facial nucleus to innervate the orbicularis oculi muscles that close the eye lid (van Ham and Yeo, 1996).

The influence of the dopamine system on the reflex blink circuit is thought to occur via inhibitory projections of the substantia nigra pars reticulata onto neurons within the superior colliculus (Basso et al., 1996). Because medium spiny neurons of the striatum receive input from and project back to the substantia nigra (Haber et al., 2000), dopamine receptors expressed on striatal neurons can directly modify activity of the substantia nigra (Akkal et al., 1996) and thereby influence reflex blinks by altering the activity of the superior colliculus, and thereby the nuclei downstream that innervate the muscles controlling eye blinks. In line with this is evidence that dopamine agonists increase blink rate (Elsworth et al., 1991) while dopamine receptor antagonists decrease blink rate (Kleven and Koek, 1996). The results of the current study, and our previous work in non-human primates, however, suggest that there is a selective relationship between spontaneous eye blink rates and D₂-like receptor density, which cannot be accounted for by these hypothesized influences of dopamine within the reflex blink circuitry. This remains an open research question, and further studies will be required to determine what role the D₂-like receptor system is playing in the eye blink response.

Utility of eye blink rate in assessing D₂-like receptor function

Although PET is one of the most highly translational tools for measuring D₂-like receptors, the labor and technical costs, as well as equipment requirements, that are necessary for such studies limit its utilization in animals, as well as humans. Here, we provide evidence that spontaneous eye blink rates in rats are altered following chronic administration of haloperidol, providing translation support for the relationship we previously detected in non-human primates that, together, indicate spontaneous eye

blink rate may serve as a non-invasive proxy of D₂-like receptors. The ability to measure spontaneous eye blinks repeatedly, with minimal equipment, and, as demonstrated here and in Chapter 4, identically across species, the spontaneous eye blink phenotype has the potential to be one of the most versatile and translational behavioral measures available for indexing the D₂-like receptor.

Disruptions in D₂-like receptor signaling, that are present in psychopathologies such as addiction, schizophrenia, and bulimia nervosa (Wong et al., 1986; Wong et al., 1997; Lee et al., 2009; Broft et al., 2012), may emerge during adolescence when the behavioral symptoms of these disorders typically first appear. *In vitro* studies examining the density of dopamine receptors at various stages of rat adolescent and adult development have suggested that D₂-like receptor density robustly increases during puberty and that these levels of the D₂-like receptor are pruned back into adulthood (Teicher et al., 1995). Thus, dysfunctional pruning of the pubertal-induced increases in D₂-like receptors may produce the elevated D₂-like receptor density that is a common phenotype in adults with schizophrenia (Wong et al., 1986). However, until now, the experimental techniques available to test this hypothesis were not feasible for the large-scale studies needed to address this hypothesis. By using spontaneous eye blink rate as a measure of D₂-like receptor function, D₂-like receptor function can be assessed at multiple developmental time points to determine whether dysfunctional pruning of the D₂-like receptors following adolescence enhances the risk of an individual to develop schizophrenia.

Similarly, longitudinal measurements of spontaneous eye blink rate may also provide insight into the mechanisms that influence the development of substance use disorders. Pre-existing low D₂-like receptor availability predicts greater cocaine-taking behaviors in animals (Nader et al., 2006; Dalley et al., 2007), but, because of the limitations of PET, this relationship has not been examined in humans. Therefore, spontaneous eye blink rates, as a proxy of D₂-like receptors, could be used to test this

hypothesis and, more importantly, potentially identify individuals who are at greater risk for developing a substance dependence problem at a point when interventions are more effective.

Conclusion

Although additional studies are needed to determine the validity of spontaneous eye blink rates as a proxy of D₂-like receptors in humans, this study and the non-human primate study provide convergent results supporting this biobehavioral association. The potential uses of spontaneous eye blink rate as a proxy of D₂-like receptors is vast, possible being a diagnostic tool for psychiatric disorders, used to test specific neurodevelopmental hypotheses regarding D₂-like receptor, as well as a high-throughput phenotype for large-scale genetic studies of D₂-like receptors. Thus, spontaneous eye blink rates can provide substantial insight into the role of D₂-like receptor dysfunction in psychiatric disorders.

Figures

Figure 5.1: A representative 10-point saturation curve for specific [^3H]spiperone binding in the dorsolateral striatum of one rat. Different concentrations of [^3H]spiperone (X axis) were incubated with tissue homogenates in the presence and absence of (+) butaclamol to determine specific [^3H]spiperone binding (Y axis).

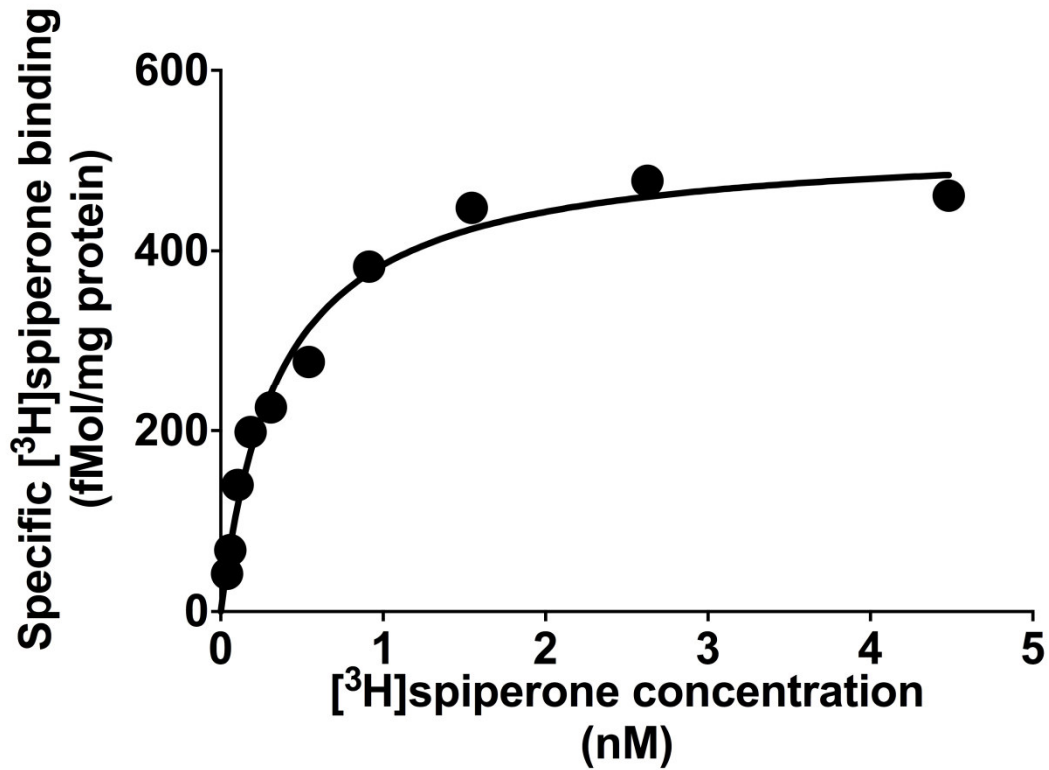


Figure 5.2: Average spontaneous eye blink rate (blinks per minute of visibility +/- SEM) in vehicle (closed circles) and haloperidol (open circles) exposed rats before (Baseline) and after the 30 d exposure period (Post-Dosing Regimen). ** p<0.01.

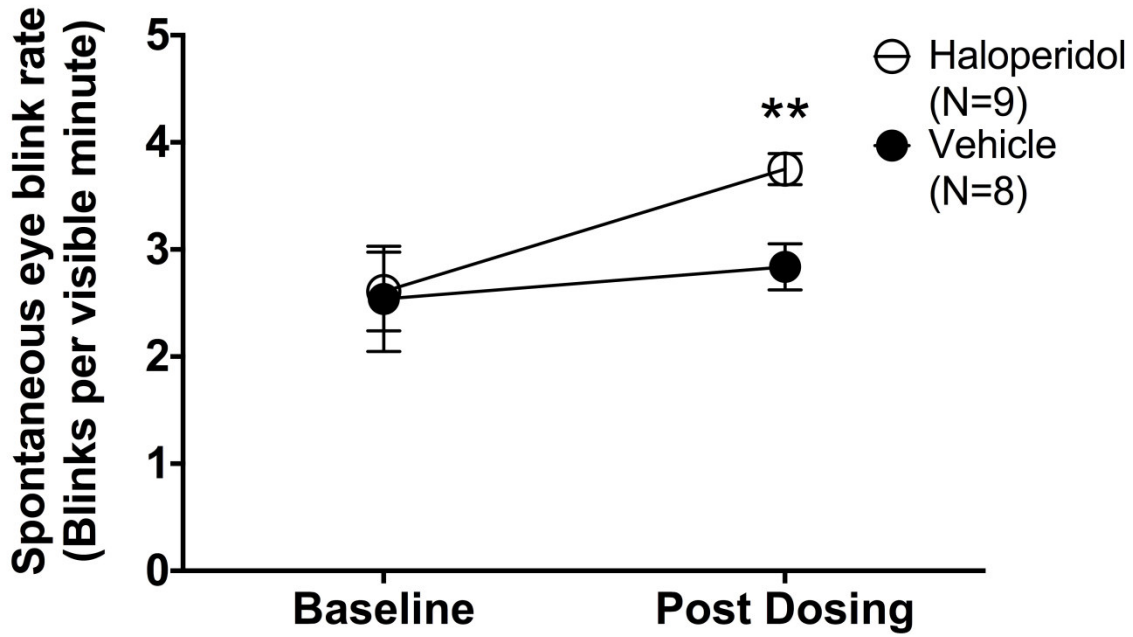


Figure 5.3: *In vitro* measurements for the saturation curves generated with [³H]spiperone. The maximal number of [³H]spiperone binding sites (B_{max}) in vehicle (N=8; closed bars) and haloperidol (N=9; open bars) exposed rats is presented in panel A and the equilibrium dissociation constant (K_d) measurements presented in panel B. Data presented as mean +/- SEM. ** p<0.01.

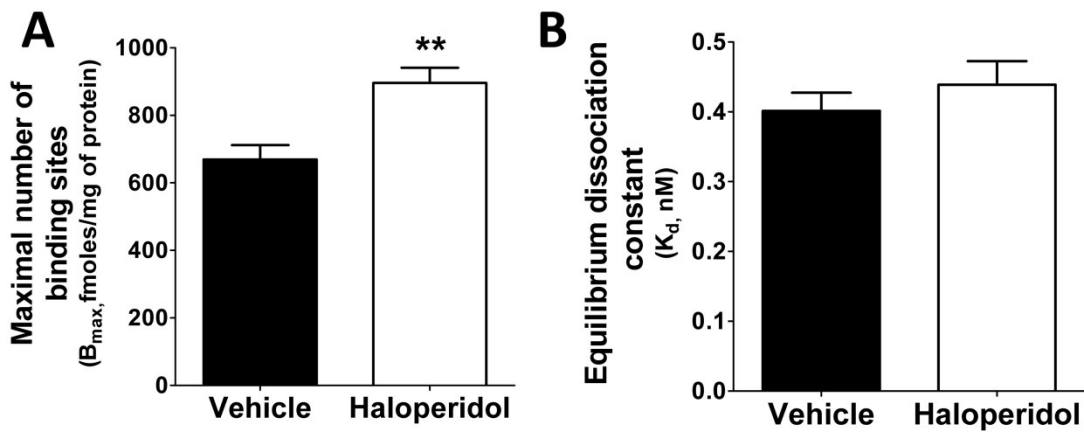
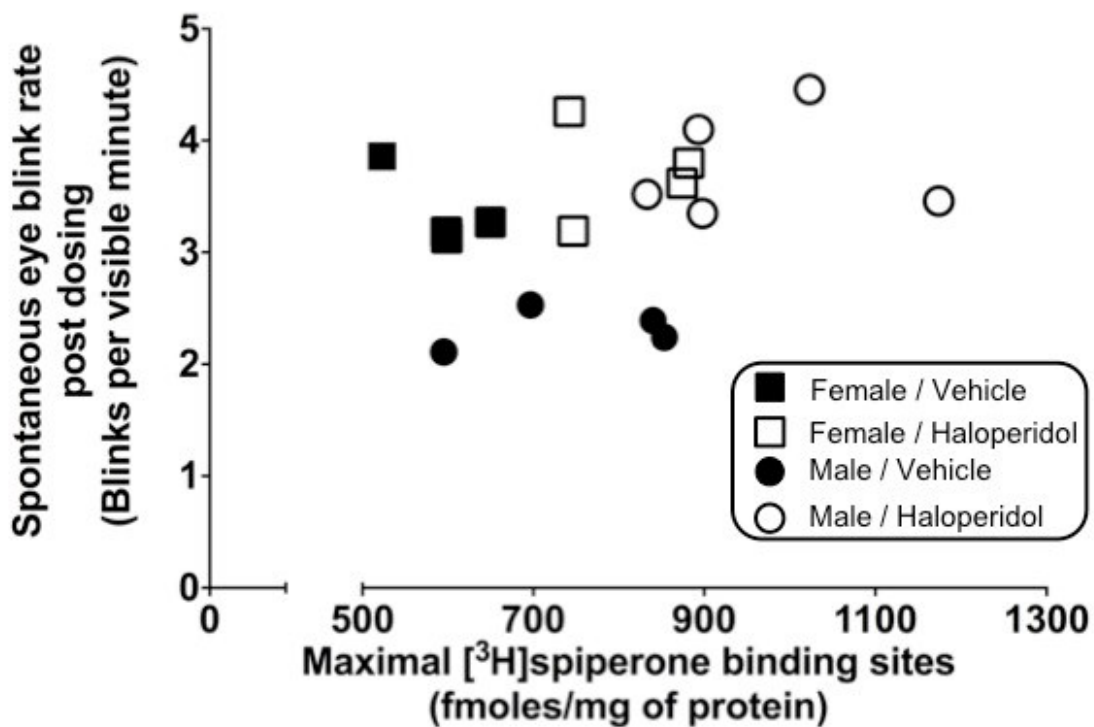


Figure 5.4: The relationship between the maximal number of [³H]spiperone binding sites (B_{max}) in the dorsolateral striatum and average spontaneous eye blink rates in female (square symbols) and male (circle symbols) rats exposed to either vehicle (dark symbols) or haloperidol (open symbols) daily for 30 days.



Chapter 6

General Discussion

Summary of findings

The multi-dimensional nature of mental disorders, characterized by a collection of symptoms that span conceptually unrelated behavioral and cognitive domains, and which most likely rely on diverse neural systems, makes the identification of disease-specific biomarkers challenging. This may be further exacerbated by the classification of individuals into categories such as “affected” or “unaffected”, based on the presence or absence of symptoms, a practice that obfuscates the natural variation in symptom dimensionality within clinical populations. Because individual differences in the severity and symptomatology within disorders may be an essential feature in understanding inter-individual differences in response to treatment, deconstructing psychiatric disorders into simpler and more refined phenotypes may provide the resolution needed to understand the genetic, cellular, and behavioral underpinnings of mental disorders (Bilder et al., 2009).

The studies included in this dissertation sought to utilize this approach in order to identify the neural mechanisms underlying inhibitory control, given that aberrant inhibitory control processes have been consistently proposed as core features of several psychiatric disorders (Floresco and Jentsch, 2011). Because the ability to inhibit maladaptive or inappropriate behaviors in favor of those with greater long-term gains is believed to be a consequence as well as contribute to the compulsive drug-seeking and taking that is characteristic of addiction (Jentsch and Taylor, 1999; Monterosso et al., 2007), these studies were directed at providing a mechanistic account for the bi-directional relationship between cognitive control and substance dependence.

Based on evidence that D₂-like receptor availability is low in a variety of substance-dependent populations (Volkow et al., 1993; Lee et al., 2009) and previous pharmacological studies implicating a

selective role of the dopamine D₂-like receptors in inhibitory control processes (Lee et al., 2007) the first study in this dissertation examined whether natural variation in D₂-like receptor availability co-varied with individual differences in the ability to inhibit a pre-potent response follow a change in stimulus-reward contingencies. We report that natural occurring variation in D₂-like receptor is correlated with the ability of monkeys to reverse a stimulus-reward association, but not with their ability to acquire or remember an association, and the sensitivity of these individuals to positive, but not negative, feedback. In the second study, we examined how chronic exposure to a realistic dosing regimen of methamphetamine altered the D₂-like receptor system and reversal learning performance to determine the impact that drugs with abuse liability have on inhibitory control processes. Chronic exposure to methamphetamine reduced D₂-like receptor availability and the degree of change in D₂-like receptor availability correlated with the change in positive-feedback sensitivity that occurred following the dosing regimen, providing evidence that the drug-induced changes in D₂-like receptor signaling have a functional impact on positive-feedback sensitivity which governs inhibitory control. In the third study, the functional relevance of changes in D₂-like receptor availability was examined by collecting neuroimaging, behavioral, pharmacology, and biochemical measurements of the D₂-like receptor all within the same subjects. We report that differences in neuroimaging measurements of the D₂-like receptor reflect biologically relevant differences in the density of D₂-like receptors and identify spontaneous eye blink rate as a non-invasive behavioral proxy of D₂-like receptors. In the last study, we examined the translational applicability of spontaneous eye blink rate as a proxy of D₂-like receptor function in rats before and after daily exposure to a D₂-like receptor antagonist. Chronic haloperidol exposure increased D₂-like receptor density and spontaneous eye blink rates, providing supporting evidence that eye blink may be a potential tool for interrogating the striatal D₂-like receptor system. The series of studies described here demonstrate that the D₂-like receptor is critically involved in the

adaptive control of behavior, providing a biobehavioral link between D₂-like receptors and behavioral control that has broad implications for understanding psychopathology.

Potential mechanisms

Although the current results demonstrate that deviations in D₂-like receptors alter cognitive control processes by influencing the ability of individuals to use positive outcomes to guide behaviors, the precise way in which this occurs is unknown. As was proposed in Chapter 3, phasic release of dopamine in response to a positive outcome (Schultz, 1997) may act on D₂-expressing medium spiny neurons to reduce the indirect pathway constraints on thalamic output to the cortex, increasing the likelihood that the same response will be made on the following trial. Low levels of D₂-like receptors, whether due to natural or pathological reasons, would impair the ability of individuals to detect these changes in dopamine levels, reducing the utilization of positive feedback to guide subsequent behaviors. Although positive feedback is involved in the acquisition and reversal of an association (Groman et al., 2011), the influence of positive-feedback sensitivity may be greatest in situations where the synaptic strength of a previously learned stimulus-response association is high, such as in a change in stimulus-reward contingencies. Decrements in the ability of positive outcomes to guide subsequent responding, through dopamine D₂-dependent mechanisms, may enable these strong, pre-existing synapses to drive behavior, impairing the ability of individuals to exert control over pre-potent behaviors

However, the ability to modify behaviors adaptively in response to environmental changes most likely relies on the coordinated activity of multiple nuclei within the corticostriatal circuit, given long-standing evidence that the prefrontal cortex, and specifically the orbitofrontal cortex, are critical to adaptive control of behavior (McEnaney and Butter, 1969; Dias et al., 1996a). Emerging studies have indicated that the striatum is also involved in this ability, demonstrating that focal lesions to the striatum can produce behavioral impairments that are similar, if not identical, to those detected

following lesions of the orbitofrontal cortex (Clarke et al., 2008). Therefore, corticostriatal brain regions may work in concert to optimize and guide behavior.

With this in mind, a variety of neurotransmitter systems within these brain regions can participate in different ways to facilitate behavior, and evidence indicates there is an anatomical dissociation between serotonin and dopamine influences on behavioral control. Depletion of serotonin, but not dopamine, in the orbitofrontal cortex impairs reversal learning performance (Clarke et al., 2004), while this neurotransmitter influence is reversed in the striatum, with depletion of dopamine, but not serotonin, impairing reversal learning performance (Clarke et al., 2011). Therefore, individual differences in monoaminergic systems within distinct brain nuclei may interact to predict inhibitory control function. Indeed, in appendix 1, we report that levels of serotonin in the orbitofrontal cortex and dopamine in the putamen interact to predict individual differences in reversal learning performance (Groman et al., 2013).

Because D₂-like, and not D₁-like, receptor availability is associated with reversal learning performance and positive-feedback sensitivity, it is likely that dopamine acting specifically through D₂-like receptors interacts with orbitofrontal serotonin systems to influence inhibitory control processes. Indeed, perturbations to striatal D₂-like receptor signaling have been reported to alter prefrontal serotonin levels (Mendlin et al., 1999) and perturbations to prefrontal serotonin receptors (5HT_{2C}) alter striatal dopamine release (Leggio et al., 2009), suggesting that there is a functional circuit between these neurochemical systems across discrete brain regions work together to optimize and guide goal-directed behavior.

Implications for other psychiatric disorders

Although the focus of these investigations has been on addiction-related disorders, the results of the studies included in this dissertation are equally applicable to other psychopathologies in which

inhibitory control processes are altered, such as attention deficit hyperactive disorder (ADHD) and bipolar disorder (Walshaw et al., 2010). Inhibitory control is an important dimension of ADHD that may influence the likelihood of these individuals to develop a drug use disorder (Groman et al., 2009). Based on the results of the studies in this dissertation, dysfunction of the D₂-like receptor may be present in individuals with ADHD, impacting self-control processes and influencing the likelihood of these individuals to develop a substance dependence problem (Lee et al., 2011b). However, as discussed in Chapter 6, D₂-like receptor measurements with PET are not commonly conducted in adolescents, due to the potential health risks associated with radioactivity, leaving this question unaddressed. However, animal models of ADHD have provided some evidence supporting this hypothesis (Fan and Hess, 2007).

Similar to the pattern of D₂ dysfunction detected in individuals with schizophrenia, D₂-like receptor availability is increased in individuals with bipolar disorder (Pearlson et al., 1995) and dysregulation of the genes encoding for the D₂ receptor have been proposed to be the initial trigger in the pathogenesis of bipolar disorder (Zhan et al., 2011), perhaps through inefficient pruning of D₂-like receptors during adolescence that may occur in schizophrenia (O'Donnell, 2010). Although D₂-like receptors are increased in these individuals, performance in tasks of cognitive control is impaired (Dickstein et al., 2010) suggesting that, similar to the relationship proposed for prefrontal D₁-like receptors and working memory, striatal D₂-like receptors and inhibitory control may be non-monotonically related. The ability to test this hypothesis has been limited by the ability to measure D₂-like receptor availability in large-samples; however, as discussed in Chapter 5, the use of spontaneous eye blink rates as a proxy of D₂-like receptors would allow large-scale phenotyping of individuals to address this question.

Implications in the treatment of psychiatric disorders

Improving D₂-like receptor function has been proposed to represent a primary treatment strategy for several psychopathologies, but most notably for addiction (Kosten et al., 2002). However, clinical trials of D₂-like receptor agonists have generally been discouraging (Soares et al., 2001). This may be because the pharmacological action of D₂-like agonists is very different from that of dopamine. Slight fluctuations in intrasynaptic dopamine concentrations, caused by changes in the phasic burst firing patterns of midbrain dopamine neurons, are thought to be detected primarily by D₂-like receptors since their affinity for dopamine is lower than that of D₁-like receptors. Thus, administration of D₂-like agonists, which tonically stimulate D₂-like receptors in a physiologically unrealistic manner, may prevent the detection of subtle changes in dopamine levels by the D₂-like receptor that are believed to encode the very prediction errors that enable learning and guide behavior (Schultz, 1997).

Thus, treatment strategies aimed at increasing D₂-like receptor density in substance-dependent individuals, rather than enhancing the function of present receptors, may prove to be a more effective treatment strategy. Although preclinical work in animals supports this hypothesis, demonstrating that viral-mediated overexpression of D₂-like receptors in the striatum reduce cocaine and alcohol self administration in rats (Thanos et al., 2004; Thanos et al., 2008), the techniques available to increase D₂-like receptor density in humans, outside of protracted abstinence, are limited. In a recent, unpublished study, striatal D₂-like receptor availability was found to increase in methamphetamine-dependent individuals following an 8-week exercise program, compared to individuals who participated in a health education program (Robertson et al., *unpublished*), suggesting that exercise may serve as a non-invasive therapeutic for enhancing D₂-like receptor density. Indeed, emerging literature demonstrating that improvements in cognitive functions following exercise in healthy and individuals with ADHD (Pieramico et al., 2012; Pontifex et al., 2013), as well as improving maintenance of smoking cessation (Marcus et al., 1995), which may be a behavioral effect due to exercise-induced enhancements in D₂-like receptor density.

Another possibility for the lack of a clear therapeutic effect of D₂-like receptor agonist is that enhancing D₂-like receptor signaling may only be effective in those individuals whose primary deficit is in inhibitory control processes. Although at a group level D₂-like receptor availability is lower in drug-dependent individuals, there is a high-degree of variability in degree of deviation in D₂-like receptor availability within these populations (Volkow et al., 1993; Volkow et al., 2001b), which has also been reported in non-human primates chronically exposed to drugs (Nader et al., 2006; Groman et al., 2012). Thus, pharmacological drugs that enhance D₂-like receptor signaling may only be effective in individuals with baseline impairments in self-control processes that are due to D₂-like receptor dysfunction (Ersche et al., 2011) while impairing inhibitory control function in individuals with normal to supranormal levels of D₂-like receptors, such as that detected in individuals with bipolar and schizophrenia (Wong et al., 1986; Pearlson et al., 1995). Thus, spontaneous eye blink rate, serving as a proxy for D₂-like receptors, may enable the degree of D₂-like receptor deficit across a variety of psychopathologies to be determined within a clinical setting to allow for implementation of biologically-based individualized treatment strategies.

Conclusion

This series of studies provides evidence for the utility of the phenomic approach for identifying and investigating the molecular basis of mental disorders. Although our focus was on inhibitory control, these same principles can be applied to investigate other domains of interest (i.e., sensory gating) at different levels (i.e. circuitry, gene expression) in order to isolate the precise pathology underlying mental disorders. By using this approach, we can facilitate an understanding of these disorders, identify unique and overlapping biomarkers for them, and provide insight into the mechanisms that can lead to the development of novel treatments.

Appendix 1

Monoamine levels within the orbitofrontal cortex and putamen interact to predict reversal learning performance

Summary

The compulsive and inflexible behaviors that are present in many psychiatric disorders, particularly behavioral addictions and obsessive-compulsive disorder, may be due to neurochemical dysfunction within the circuitry that enables goal-directed behaviors. Experimental removal of serotonin or dopamine within the orbitofrontal cortex or dorsal striatum, respectively, impairs flexible responding in a reversal learning test, suggesting that these neurochemical systems exert important modulatory influences on goal-directed behaviors. Nevertheless, the behavioral impairments present in psychiatric disorders are likely due to subtle neurochemical differences, and it remains unknown whether naturally-occurring variation in neurochemical levels associate with individual differences in flexible, reward-directed behaviors. The current study assessed the ability of 24 individual juvenile monkeys to acquire, retain and reverse discrimination problems and examined whether monoamine levels in the orbitofrontal cortex, caudate nucleus and putamen could explain variance in behavior. The interaction between dopamine levels in the putamen and serotonin levels in the orbitofrontal cortex explained 61% of the variance in a measure of behavioral flexibility, but not measures of associative learning or memory. The interaction mirrored that of a hyperbolic function, with reversal learning performance being poorest in either monkeys with relatively low levels of orbitofrontal serotonin and putamen dopamine or in monkeys with relatively high levels of orbitofrontal serotonin and putamen dopamine levels. These results support the hypothesis that subcortical and cortical neuromodulatory

systems interact to guide aspects of goal-directed behavior, providing insight into the neurochemical dysfunction that may underlie the inflexible and compulsive behaviors present in psychiatric disorders.

Introduction

Adaptive modulation of behavior in response to environmental change is necessary in order for individuals to behave in both a flexible and goal-directed manner. Conversely, the compulsive and rigid behaviors that are present in some individuals with behavioral addictions or obsessive-compulsive disorder may result from impairments in the neural circuitry that normally allows individuals to flexibly update their behaviors. Consistent with this hypothesis, affected individuals exhibit deficits in laboratory tasks of behavioral flexibility (Ersche et al., 2008; Valerius et al., 2008; Ghahremani et al., 2011), as well as abnormalities in brain regions that are critical for flexible behavior (Thompson et al., 2004; Chamberlain et al., 2008).

Behavioral flexibility can be assessed across species using reversal learning procedures, which index an individual's ability to adaptively modify behavior when previously learned reward associations are altered. The orbitofrontal cortex has been well established as a region critical to flexible, adaptive responding, as lesions to the orbitofrontal cortex impair the extinction of a previously reinforced response (Butter et al., 1963) and the ability to modify behaviors in a reversal-learning task (Dias et al., 1996b; Fellows and Farah, 2003). In addition, efficient updating of behavior during reversal in normal humans is associated with event-related recruitment of the lateral orbitofrontal cortex (Ghahremani et al., 2010).

The orbitofrontal cortex is connected with both the caudate nucleus and putamen (Selemon and Goldman-Rakic, 1985) (Haber et al., 2006), through which it likely acts to alter cognitive and motor behaviors. The striatum has long been recognized as an important brain region for simple forms of goal-directed behaviors (Yin et al., 2005; Yin et al., 2006), and recent evidence has indicated that the dorsal striatum is also involved in reversal learning (Divac et al., 1967; Clarke et al., 2008; Castane et al., 2010). Moreover, functional neuroimaging studies in humans have demonstrated activation within the caudate nucleus and putamen during performance of a reversal learning task (Freyer et al., 2009; Ghahremani et

al., 2010), suggesting that the ability to adaptively modify behaviors may be governed by an integrative network involving both cortical and subcortical brain nuclei.

Within each of the brain regions implicated in reversal learning, a variety of neurotransmitters can participate in different ways to facilitate behavior. Neurotransmitter depletion studies indicate that within the orbitofrontal cortex, serotonin plays an essential role, while dopamine does not (Clarke et al., 2004). On the other hand, within the dorsal striatum, depletion of dopamine, but not serotonin, impairs reversal learning performance (Clarke et al., 2011), a finding consistent with reported deficits in behavioral flexibility in Parkinson's disease (Peterson et al., 2009).

Although experimental depletion of dopamine and serotonin in these brain regions can alter reversal learning performance, it remains unknown whether naturally-occurring variation in monoamine levels explains individual differences in behavioral flexibility. Because the deficits in behavioral flexibility associated with neuropsychiatric disorders are likely linked to subtle, heritable patterns of neurochemical anomalies, it remains crucial to identify whether normal variation in behavioral abilities are linked with normal variation in neurotransmitter function. One approach for determining the neurochemical basis of cognition is to relate differences in neurochemical measurements with differences in behavior at an individual level (Sahakian et al., 1985). We have previously shown that naturally-occurring variation in striatal D₂-like receptor availability is associated with individual differences in reversal-learning performance (Groman et al., 2011), suggesting that slight differences in neurotransmitter function may contribute to individual variability in behavioral flexibility. Furthermore, the experimental depletion studies were conducted in independent studies and did not examine the possibility that neuromodulatory systems within discrete brain regions functionally interact with each other to influence the neural circuitry that guides behavior.

The current study assessed a cohort of juvenile monkeys for their ability to acquire, retain and reverse novel visual discrimination problems, and their brain monoamine levels were subsequently ascertained. We used these data to test a specific hypothesis: namely, whether the interaction between serotonin levels in the orbitofrontal cortex and dopamine levels in the putamen and caudate nucleus predicted behavioral performance. Specifically, we hypothesized that the interaction between levels of dopamine in the dorsal striatum and serotonin in the orbitofrontal cortex would predict performance during reversal learning, but not the ability to acquire or retain visual discrimination problems.

Methods

Subjects: 24 male juvenile vervet monkeys (*Chlorocebus aethiops sabaues*) born at the UCLA Vervet Research Colony (VRC) were involved in this study. The subjects were recruited from two successive annual birth cohorts (12 from the 2004 cohort and 12 from the 2005 cohort); all subjects were housed in peer groups during the period of testing which began at 2 yrs of age and ended by 3 yrs of age. Subjects had unlimited access to water and received twice daily portions of standard monkey chow (Teklad). Chow was never withheld or reduced to motivate performance of the tasks.

All monkeys were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Discrimination acquisition, retention and reversal learning: Subjects were assessed for their ability to acquire, retain and reverse visual discrimination problems, using procedures similar to those previously described (Groman et al., 2011; Groman et al., 2012). Testing was conducted 6-7 days per week. Monkeys were trained to enter into a metal tunnel which was fitted with a modified Wisconsin General Testing Apparatus. This apparatus was equipped with an opaque screen that could be raised or lowered to present three equally spaced opaque boxes to the monkey. These boxes were fitted hinged lids, that allowed for small pieces of food reward (i.e. a piece of banana, apple or grape) to be concealed within, and inserts on the lid where unique visual stimuli could be displayed (clip art).

Testing sessions began when the opaque screen was raised to present the three boxes (each fitted with a unique visual stimulus) to the monkey. One of the three visual stimuli was uniformly associated with food reward, while the others were not. Monkeys were only allowed to open one box per trial. A trial ended after a correct choice (opening the box containing the food reward), an incorrect

choice (opening an empty box), or an omission (no response for 30 s) occurred. The position of the visual stimuli was pseudo-randomly varied across trials. Subjects received up to 30 trials per day.

Initially, subjects were trained to acquire four consecutive discrimination problems, each involving three novel visual stimuli. Subjects were required to learn which one of the three stimuli was associated with food reward by achieving a performance criterion (8 correct choices within 10 consecutive trials). If the performance criterion was not achieved in 30 trials, the session was terminated and the monkey was returned to the social enclosure. The same discrimination problem was presented the following day(s) until the performance criterion was met.

After completing the four discrimination-acquisition problems, monkeys were then trained to acquire, retain and reverse novel discrimination problems. The first phase of each discrimination problem was an acquisition phase which was identical to the acquisition-only training the monkeys had previously received. Once the same performance criterion had been met, the session was terminated and the monkey returned to the social enclosure.

One day after reaching criterion for acquisition, the ability of subjects to remember the stimulus-reward association from the day prior was assessed in a retention phase, where the stimulus-reward contingencies remained unchanged relative to the previous training day. This phase persisted until subjects met the performance criterion of 4 correct responses in 5 consecutive trials. Immediately after reaching the criterion in the retention phase, the reversal phase of the discrimination problem began; there were no environmental events that cued this transition, other than a change in reinforcement contingencies. In the reversal phase, the stimulus that was previously rewarded was no longer rewarded, and one of the two previously unrewarded stimuli was now rewarded. The reversal phase persisted until monkeys achieved criterion (8 correct choices in 10 consecutive trials) or until 30 trials had been completed, whichever occurred first. If monkeys did not reach the performance criterion

within 30 trials, the reversal phase continued the following day(s) until the performance criterion was met. Monkeys were assessed on their ability to acquire, retain and reverse three novel discrimination problems. Due to inclement weather that prevented testing, one reversal session was not completed by four different monkeys, and these data were excluded from the analysis.

The primary dependent measures were the total number of trials required to reach criterion and the number of correct responses made in the acquisition, retention and reversal phases. For the reversal phase, the total number of responses directed at the previously rewarded stimulus (a perseverative response) and the never rewarded stimulus (a neutral response) were also recorded. The proportion of each response type (correct, perseverative, or neutral) was determined by dividing the total number of each response by the number of trials required to reach criterion for each phase.

Quantification of monoamine levels: Approximately 2-3 months after completing these behavioral assessments, brain tissue was collected at necropsy, using methods previously described (Jentsch et al., 1997; Groman et al., 2012). Tissue homogenates were assayed using high pressure liquid chromatography similar to procedures described elsewhere (Jentsch et al., 1997) and protein content in homogenates was quantified using the Lowry method (Lowry et al., 1951).

Tissue was acquired from a wide range of brain regions. However, we restricted our analyses here to monoamine levels in the orbitofrontal cortex, putamen and caudate nucleus collected from regions identified in Figure 1. Based on previous evidence (Haber et al., 2006), tissue acquired from the caudate nucleus and putamen were within the terminal fields of the orbitofrontal and dorsal anterior cingulate cortex. Although behavioral data was collected in 24 monkeys, brain tissue was only collected from 20, as 4 subjects were transferred to another behavioral protocol.

Statistical analyses: All statistical analyses were conducted using SPSS 15.0. The reliability of the behavioral measurements was determined using Cronbach's α , a coefficient of reliability. Linear

regressions using cohort (2004 vs. 2005) as an independent variable were then conducted on all measurements prior to any further analysis to remove extraneous variance that was attributable to cohort. These residuals were then centered for further statistical analysis. To examine the relationship between monoamine levels and behavior measurements, multiple linear regression was conducted with individual monoamine levels and their interaction term(s) as the independent variable(s) and the behavioral measurement(s) as the dependent variable using a forced entry model:

$$\text{Behavior} = \theta_0 + \theta_1(\text{putamen (or caudate) dopamine levels}) + \theta_2(\text{orbitofrontal serotonin levels}) + \theta_3(\text{putamen (or caudate) dopamine levels} * \text{orbitofrontal serotonin levels}) + \varepsilon$$

Results

Acquisition, Retention and Reversal Learning: Reliability of the number of trials required to reach criterion across the acquisition and reversal sessions was moderately high (acquisition: Cronbach's $\alpha=0.72$, reversal: Cronbach's $\alpha=0.62$). However, contrary to our previous observations, reliability of the number of trials required to reach criterion across the three retention sessions was surprisingly low (Cronbach's $\alpha=0.33$). The subjects required fewer trials to reach criterion in the acquisition phase (56.2 +/- 28.6) compared to the reversal phase (78.2 +/- 38.7) ($t(22)=-4.93$; $p<0.001$), indicating that they found the reversal of the stimulus-reward contingencies more difficult than their initial acquisition. Descriptive statistics for the error type in the reversal phase revealed that the probability of making a response to the initially reinforced stimulus was significantly greater than the probability of making a response to the never-rewarded stimulus ($t(23)=15.58$; $p<0.001$), showing that the increase in the number of trials required to reach criterion during reversal was driven by an increased probability of making a perseverative response, rather than neutral responses.

Monoamine Levels: The average levels of dopamine and standard error, normalized to protein content, in the putamen and caudate nucleus were 101 +/- 13.2 ng/mg and 110 +/- 5.58 ng/mg, respectively. The average level of serotonin and standard error within the orbitofrontal cortex, normalized to protein content, was 1.94 +/- 0.29 ng/mg.

Neurochemical Correlates of Behavior: Multiple linear regression was used to examine the relationship between serotonin and dopamine levels in the orbitofrontal cortex and putamen, respectively, and the behavioral measures collected. First, the relationship between orbitofrontal serotonin levels, putamen dopamine levels and their interaction was examined with respect to the average number of trials required to reach criterion in the acquisition, retention and reversal phases. Neither levels of dopamine in the putamen, serotonin in the orbitofrontal cortex nor the interaction of the two predicted a significant amount of variance in either acquisition or retention performance (acquisition: $F_{3,14}=1.97$,

$p=0.16$; retention: $F_{3,11}=0.079$, $p=0.97$) (Figure 2A and 2B). Moreover, the main effects of orbitofrontal serotonin and putamen dopamine levels did not predict a significant proportion of variance in the number of trials required to reach criterion in the reversal phase ($F_{2,15}=0.74$; $p=0.49$). However, as hypothesized, the regression model including the interaction term was significant (R^2 change $F_{1,14}=18.82$; $p=0.001$), accounting for 61% of the variance in the average number of trials required to reach criterion in the reversal phase ($F_{3,11}=7.36$; $p=0.003$). In this model, the regression coefficients for orbitofrontal serotonin and putamen dopamine levels remained non-significant (all t 's < 1.5), while the regression coefficient for the interaction was significant ($t(11)=4.34$; $p=0.001$), explaining 52% more of the variance than did the main effects of dopamine and serotonin levels. The interaction regression coefficient was positive, such that as levels of dopamine in the putamen increased by one standardized unit, the slope of the relationship between orbitofrontal serotonin and number of trials required to reach criterion during reversal learning increased by a factor of 0.74. Monkeys with the lowest dopamine and serotonin levels required the greatest number of trials to reach criterion in the reversal. Amongst monkeys with low dopamine levels in the putamen (i.e. one standard deviation below the mean), relatively higher levels of orbitofrontal cortical serotonin were associated with fewer trials required to reach criterion (better performance). However, high putamen dopamine levels (i.e. one standard deviation above the mean) were associated with a positive relationship between serotonin and reversal phase trials: for monkeys with the highest putamen dopamine levels, low serotonin was associated with good reversal learning performance (Figure 2C). Therefore, the interaction between orbitofrontal serotonin and putamen dopamine levels on reversal performance mirrored that of a hyperbolic function, with reversal learning performance being poorest in either monkeys with relatively low levels of orbitofrontal serotonin and putamen dopamine or in monkeys with relatively high levels of orbitofrontal serotonin and putamen dopamine levels.

Next, we sought to determine the neurochemical and anatomical specificity of the relationships mentioned above with reversal performance. To do this, levels of serotonin in the putamen, dopamine in the orbitofrontal cortex and the interaction of the two were regressed against the average number of trials required to reach criterion in the reversal phase. This model did not account for a significant proportion of variance in reversal performance ($F_{3,14}=0.997$; $p=0.42$) (Figure 3A), providing evidence for the selective neurochemical involvement of dopamine in the putamen and serotonin in the orbitofrontal cortex with reversal learning.

Finally, levels of dopamine in the caudate nucleus, serotonin in the orbitofrontal cortex and their interaction were regressed against reversal learning performance. Contrary to our hypothesis, the regression model failed to account for a significant proportion of variance in reversal learning performance ($F_{3,14}=0.34$; $p=0.80$) (Figure 3B).

Discussion

The results of the current study demonstrate that individual differences in monoaminergic systems within distinct brain nuclei interact to predict flexibility of behavior during a reversal learning task. By contrast, these neurochemical measures did not predict basic associative learning or memory (acquisition or retention) performance. Importantly, the relationship between serotonin in the orbitofrontal cortex, dopamine in the putamen and reversal learning was highly specific because a variant model, which included dopamine in the orbitofrontal cortex and serotonin in the putamen as predictors, did not explain a significant amount of variance in reversal performance. Finally, the finding that levels of dopamine in the putamen, and not in a region of anatomical proximity (the caudate nucleus), modified the relationship between orbitofrontal serotonin and reversal learning performance, implicates anatomical specificity of the observed relationships.

Neurochemical interactions within discrete brain regions predict reversal learning performance

Previous studies have found that depletion of serotonin in the orbitofrontal cortex or dopamine in the dorsal striatum can cause reversal learning deficits (Clarke et al., 2004; O'Neill and Brown, 2007; Clarke et al., 2011). In these studies, large and systematic reductions in local neurotransmitter levels were produced. However, recently individual differences in methylphenidate-induced dopamine release were reported to correlate with methylphenidate-induced changes in reversal learning performance, indicating that dopaminergic differences, an order of magnitude smaller than depletion studies, within the striatum may directly influence reversal learning performance (Clatworthy et al., 2009). Building upon these results, the current study provides evidence that orbitofrontal serotonin levels and putamen dopamine levels functionally interact, explaining 61% of the variance in individual reversal learning performance. The interaction followed a hyperbolic function: in monkeys with low putamen dopamine levels, poor reversal learning was associated with relatively low orbitofrontal cortex serotonin; however, as dopamine levels in the putamen *increased*, the relationship between serotonin and reversal learning

reversed, with low levels of serotonin predicting relatively better reversal learning performance. The selective relationship between levels of orbitofrontal serotonin and putamen dopamine on reversal learning performance are likely linked to the increases in perseverative responding that occur during the reversal phase of the task, suggesting that a functional circuit between these monoamine systems may underlie the ability of individuals to inhibit habitual, pre-potent responses.

The current study did not detect a significant interaction between dopamine levels in the caudate nucleus, orbitofrontal serotonin and reversal learning performance, which apparently contrasts with the effects of depletion of dopamine in the dorsomedial striatum on reversal learning (O'Neill and Brown, 2007; Clarke et al., 2011). Neuroimaging studies in humans have found increased activation during reversal learning in both the putamen (Freyer et al., 2009) and in the caudate (Ghahremani et al., 2010). Moreover, individual differences in reversal learning performance in monkeys has been associated with PET-based measurements of dopamine D₂-like receptors in both the caudate nucleus and putamen (Groman et al., 2011), indicating that dopaminergic signaling in both striatal regions is important for flexible, goal-directed behaviors. Because the prefrontal cortex broadly innervates the striatum (Haber, 2003; Haber et al., 2006), dopamine within the caudate nucleus may interact with serotonin tone in other prefrontal regions, not investigated here, that are involved in goal-directed behaviors, such as the anterior cingulate cortex (Chudasama et al., 2012), and these cortical-striatal circuits work in parallel to encode discrete types of information that together guide goal-directed behavior. Of additional note, our neurochemical assessments were made in a specific OFC region (area 47), though past lesion studies examining effects on reversal learning have involved other or additional OFC regions. Given the potential functional heterogeneity of the OFC, it is possible that the pattern of results we report here are specific to area 47, with different results found (for example) in more ventromedial OFC regions often implicated in reversal performance (Butter et al., 1963; Iversen and Mishkin, 1970; Rudebeck and Murray, 2008). Systematic investigation into the neurochemical

mechanisms within the various OFC subregions is needed and may provide insight into how these neurochemical systems interact with discrete striatal compartments to guide behavior.

Potential links between putamen dopamine and orbitofrontal serotonin

Although the mechanistic nature of the interaction between orbitofrontal serotonin and putamen dopamine systems on reversal learning is unknown, there is evidence to support the idea that striatal dopamine signaling, working through D₂-like receptors, mediates evoked prefrontal serotonin release. Specifically, pharmacological blockade of striatal D₂-like receptors significantly attenuates tail-pinch evoked increases in prefrontal and striatal serotonin efflux (Mendlin et al., 1999). Therefore, dopaminergic stimulation of D₂-like receptors within the striatum may directly influence serotonin release within the orbitofrontal cortex, potentially explaining the interaction between these two neurochemical systems on tasks of behavioral flexibility.

Conversely, prefrontal serotonin system may exert a top-down influence over striatal dopamine release. Pharmacological activation of serotonin 2C receptors within the prefrontal cortex potentiates cocaine-induced striatal dopamine release (Leggio et al., 2009), implicating a facilitatory pathway between prefrontal serotonin and striatal dopamine. Based on these data, serotonin within the orbitofrontal cortex and dopamine within the putamen may coalesce at the network level, directly influencing one another, explaining the phenomena reported here. However, the direction of the interaction between dopamine tone in the striatum and serotonin levels in the orbitofrontal cortex on reversal learning performance remains unknown.

Implications for psychiatric disorders

Neurochemical dysfunction is linked with many of the behavioral impairments that are present in individuals diagnosed with addictions. For example, striatal dopaminergic dysfunction in substance

dependent individuals has been found to associate with self-report measures of impulsivity, levels of self-reported craving for drugs, and cognitive abilities (Lee et al., 2009). Notably, dopaminergic alterations within the striatum that occur in response to chronic drug exposure have been found to correlate with drug-induced changes in reversal learning performance (Groman et al., 2012). Variation in central serotonin systems also associates with impulsiveness, and pharmacological manipulation of cortical serotonin transmission signaling alters reversal learning performance (Boulougouris and Robbins, 2010; Brigman et al., 2010).

The results of the current study indicate that levels of serotonin in the orbitofrontal interact with dopaminergic tone in the putamen to influence reversal learning performance. Therefore, in different individuals, the neurochemical etiology of reversal learning deficits may be slightly different, despite the revealed behavioral deficit being identical, and correspondingly, the pharmacological treatments targeted to improve behavioral flexibility would therefore differ. These data support the notion that pharmacological treatment strategies may achieve greater efficacy if they take into account, and are targeted at remediating, an individual's particular set of regionally-specific neurotransmitter system abnormalities, each of which may exhibit complex cross-system interactions that moderate both the behavior itself and an individual's response to pharmacological intervention.

These data also support the notion that, while experimental manipulations that produce extreme changes in a neurochemical system (e.g., depletion) can reveal the necessary influences of individual transmitters on behavioral processes, additional methods may be required in order to reveal the subtle interactions between neurotransmitters that emerge at a network level, each of which may be both complex and multifaceted. Further studies of the relationship between individual differences in brain neurotransmitter phenotypes and behavior may help to decompose the role for distinct and interacting neuromodulatory systems in aspects of cognition.

Figures

Figure 1: A depiction of the brain regions collected for quantification of monoamine levels. The shaded area in **(A)** (predominately Brodmann area 47) represents the area of the orbitofrontal cortex extracted (Bregma 13.50 mm; interaural 35.40 mm). Tissue punches from the caudate nucleus and putamen (represented by the gray circles) were collected from tissue slices similar to that presented in **(B)** (Bregma 2.70 mm; interaural 24.60 mm). (Reprinted from Paxinos *et al.*, with permission from Elsevier, copyright 2009.)

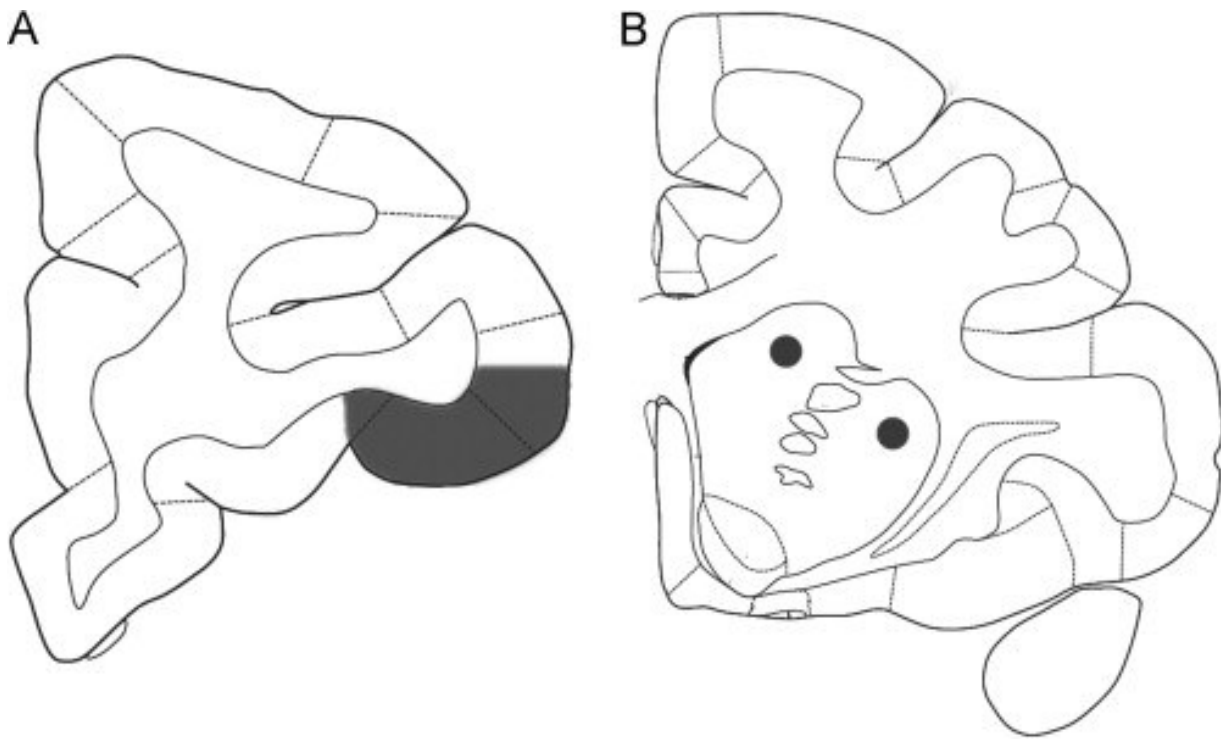


Figure 2: A three-dimensional plot of the relationship between standardized levels of dopamine in the putamen, standardized levels of serotonin in the orbitofrontal cortex, and standardized behavioral performance measures in the acquisition (A), retention (B), and reversal (C) phases. Values for individual monkeys are presented in closed circles. The transparent plane overlaid on the individual data points represents how the interaction between levels of dopamine in the putamen and serotonin in the orbitofrontal cortex predicts the number of trials required to reach criterion in each of the phases of the task. Relatively high trials to reach criterion is indicative of poorer performance in each stage.

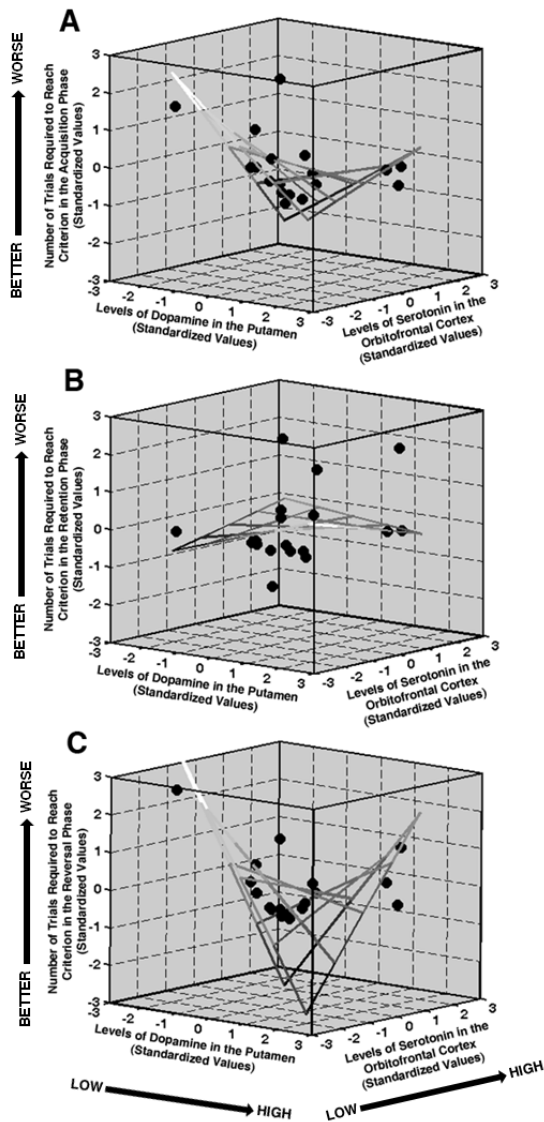
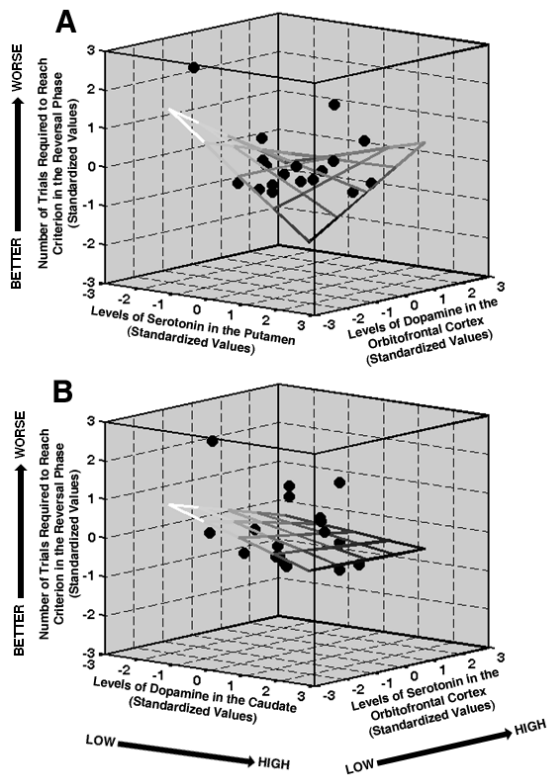


Figure 3: A three-dimensional plot of the relationship between prefrontal and striatal neurochemical levels on reversal learning performance. Values for individual monkeys are presented in closed circles. The transparent plane overlaid on the individual data points represents how the interaction between levels of monoamines predict the number of trials required to reach criterion in the reversal phase. (A) presents the relationship between standardized levels of serotonin in the putamen, dopamine in the orbitofrontal cortex, and reversal learning performance. (B) presents the relationship between standardized levels of dopamine in the caudate nucleus, serotonin in the orbitofrontal cortex, and reversal learning performance. Relatively high trials to reach criterion is indicative of poorer performance in each stage.



Appendix 2

Methamphetamine-induced increases in putamen gray matter associate with inhibitory control

Summary

Problematic drug use is associated with difficulty in exerting self-control over behaviors, and this difficulty may be a consequence of atypical morphometric characteristics that are exhibited by drug-experienced individuals. The extent to which these structural abnormalities result from drug use or reflect neurobiological-risk factors that predate drug use, however, is unknown. To determine how methamphetamine affects corticostriatal structure and how drug-induced changes relate to alterations in inhibitory control. Structural magnetic resonance images and positron emission tomography (PET) scans, assessing dopamine D₂-like receptor and transporter availability, were acquired in monkeys trained to acquire, retain and reverse three-choice visual discrimination problems before and after exposure to an escalating dose regimen of methamphetamine (or saline, as a control). Voxel-based morphometry was used to compare changes in corticostriatal gray matter between methamphetamine and saline exposed monkeys. The change in gray matter before and after the dosing regimen was compared to the change in the behavioral performance and in dopaminergic markers measured with PET. Methamphetamine exposure, compared to saline, increased gray matter within the right putamen. These changes were positively correlated with changes in performance of methamphetamine-exposed monkeys in the reversal phase, and were negatively correlated with alterations in D₂-like receptor and DAT availability. The results provide the first evidence that exposure to a methamphetamine dosing regimen that resembles human use alters the structural integrity of the striatum and that gray-matter abnormalities detected in human methamphetamine users are due, at least in part, to the pharmacological effects of drug experience.

Introduction

Substance use disorders are associated with cognitive abnormalities that are thought to reflect, in part, neurobiological dysfunction caused by long-term exposure to drugs of abuse. This view is supported by observations that exposure of animals to drugs can produce cognitive and neural alterations that resemble those that are exhibited by substance-dependent humans (Jentsch et al., 2002; Nader et al., 2006; Porter et al., 2011; Groman et al., 2012; Porter et al., 2012). The coupling of neural changes following drug exposure with alterations in cognition (Groman et al., 2012) underscores the functional impact of such changes on behavior.

Of the drug-induced effects on cognition, impairment in the ability to exert inhibitory control over behavior is the topic of much research (Izquierdo and Jentsch, 2012). Difficulty with stopping or withholding behaviors has been proposed to be a central feature of substance dependence (Jentsch and Taylor, 1999), and emerging evidence indicates that the relationship between inhibitory control and substance dependence is bi-directional. Specifically, pre-existing differences in inhibitory control predict future drug-taking behaviors (Dalley et al., 2007; Diergaarde et al., 2008; Perry et al., 2008; Anker et al., 2009), and chronic exposure to drugs of abuse can cause inhibitory control impairments to emerge (Jentsch et al., 2002; Schoenbaum et al., 2004; Schoenbaum and Setlow, 2005), indicating that inhibitory control deficits are both a cause and consequence of substance dependence (Groman and Jentsch, 2013a). Further, variability in the degree of inhibitory-control impairments exhibited by substance-dependent individuals has been reported to predict measures of sobriety (Aharonovich et al., 2006; Turner et al., 2009), suggesting that improving inhibitory control, through behavioral or pharmacological mechanisms, may serve as effective intervention and treatments for substance dependence (Groman and Jentsch, 2011b). One type of inhibitory control involves the ability to modify behavior adaptively when contingencies change, and lesions to the prefrontal cortex impair this ability in humans and animals (Dias et al., 1996a; Fellows and Farah, 2003; Rygula et al., 2010), suggesting that the prefrontal

cortex is critical for the maintenance of flexible, goal-directed behaviors (Clarke et al., 2004). Indeed, individual differences in gray-matter volume within the prefrontal cortex are related to performance on tasks of inhibitory control in healthy humans and monkeys (Haldane et al., 2008; Tabibnia et al., 2011; Sridharan et al., 2012). The dorsal striatum also plays a critical role in inhibitory control processes (Bellebaum et al., 2008; Castane et al., 2010; Clarke et al., 2011), suggesting that inhibitory control relies on an integrated network of nuclei within the corticostriatal system.

Inhibitory control deficits exhibited by stimulant-dependent individuals may reflect dysfunction in corticostriatal circuitry (Baicy and London, 2007). Consistent with this hypothesis, on average, samples of methamphetamine-dependent research participants have smaller gray-matter volumes in the prefrontal cortex (Thompson et al., 2004; Morales et al., 2012) and larger gray-matter volumes in the striatum (Chang et al., 2005; Jernigan et al., 2005), relative to methamphetamine-naive controls. While recent evidence suggests that some differences in gray-matter volume between stimulant abusers and healthy individuals are also detected in unaffected siblings of stimulant abusers (Ersche et al., 2012), no studies to date have examined whether exposure to methamphetamine causally alters gray matter in corticostriatal circuitry and whether these changes have a functional impact on measures of inhibitory control.

In order to address these questions, the current study was performed using voxel-based morphometry to measure gray matter structure in monkeys trained to acquire, retain and reverse visual discrimination problems before and after a 31-day escalating dose-regimen of methamphetamine (or saline) administration. Based on the available evidence, we hypothesized that exposure to methamphetamine would decrease gray matter within the prefrontal cortex, but increase gray matter within the striatum. Further, we expected that the changes in gray matter would be correlated with changes in reversal learning performance and dopaminergic markers in the same animals reported previously (Groman et al., 2012).

Methods

Subjects: Fourteen adult, male vervet monkeys (*Chlorocebus aethiops sabaesus* from the UCLA Vervet Research Colony), between the ages of 5 to 9 years of age, were included in the current study. The monkeys were housed in a climate-controlled vivarium, where they had unlimited access to water and received twice-daily portions of standard monkey chow in amounts that exceeded their nutritional needs (Teklad, Harlan Laboratories). Monkeys received half of their daily portion of chow after behavioral testing (~1100 hr) and the other half in the afternoon (~1500 hr). The total amount of chow was never reduced during the experiment to increase motivation for task performance. All monkeys were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education, and Welfare Publication No. (NIH) 85-23, revised 1996. The research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Drugs and Dosing Regimen: Methamphetamine hydrochloride was purchased from Sigma-Aldrich (St Louis MO). Doses of methamphetamine were prepared fresh daily in 0.9% saline and were sterile-filtered prior to administration. Injections were administered intramuscularly at a volume of 0.1 ml/kg. The dosing regimen was designed to model the escalation in both frequency of intake and cumulative daily dose reported by human users of methamphetamine (Han et al., 2011). Methamphetamine was initially administered once per day at 0.1 mg/kg, but the dose and frequency escalated across the 31 d period, with the final dose of 1.0 mg/kg being administered four times per day. Details of the dosing regimen are provided in Table 1. The initial daily dose was administered at 0830 h. During week two, a second daily dose was administered at 1630 h. During weeks three through five, the second daily dose was administered at 1330 h, and a third daily dose given at 1630 h. For the last two weeks of treatment, the second daily dose was administered at 1100 h, the third at 1330 h, and a fourth at 1600 h.

Discrimination Acquisition, Retention and Reversal Learning: As previously described in detail (Groman and Jentsch, 2011b; Groman et al., 2012), subjects were trained to acquire, retain and reverse visual discrimination problems in a modified Wisconsin General Testing Apparatus. On each trial, monkeys were allowed to open a single box. A trial ended when the subject made a correct response to rewarded stimulus (rewarded with a small piece of fruit), an incorrect response, or an omission occurred (no response for 2 min), and a 20-sec inter-trial interval followed. The spatial position of the different visual stimuli was varied pseudorandomly across trials.

Each discrimination problem consisted of three phases: acquisition, retention and reversal. In the acquisition phase, subjects were required to learn which one of the three visual stimuli was associated with reward solely through feedback provided by the task. Once the performance criterion was met (seven correct choices within 10 consecutive trials), the session was terminated and the monkey returned to the home cage. If the monkey did not reach the performance criterion within 80 trials, the session ended and the same discrimination problem presented the following day(s) until the performance criterion was met. One day after reaching criterion, subjects were assessed in the retention phase, where the stimulus-reward contingencies remained unchanged. Immediately after reaching the performance criterion (four correct responses within five consecutive trials), un signaled to the monkey, the reversal phase began. In the reversal phase, the stimulus-reward contingencies changed such that the previously rewarded stimulus was no longer rewarded and one of the two previously non-rewarded stimuli was rewarded. The reversal phase continued until the performance criterion was met (seven correct choices within 10 consecutive trials) or until 80 trials had been completed, whichever occurred first. The number of trials required to reach criterion in the reversal phase was the primary dependent measure.

Subjects were trained on several novel discrimination problems before and after the 31-day dosing regimen, each using novel stimuli. Drug effects on behavioral performance were determined by

comparing data at three time points: (1) behavioral performance of subjects in the discrimination problem completed immediately prior to beginning the dosing regimen referred to as the “baseline assessment”; (2) performance during 3 weeks within the course of the treatment regimen (referred to as the “3 week assessment”; to limit the anorectic effects of methamphetamine, this test occurred after at least 36 h had elapsed since the last methamphetamine or saline administration); and (3) performance at 5 d after the last drug administration (referred to as the “5 d post-exposure assessment”). The baseline data for 12 of the 14 monkeys used in the current study have been reported (Groman et al., 2011), as were the behavioral data before and after the dosing regimen (Groman et al., 2012).

After completion of the dose regimen, two additional behavioral assessments were conducted, but the performance criterion was increased (nine correct choices in 10 completed trials for the acquisition, retention, and reversal phases) to augment the cognitive demands of the task. Specifically, the additional acquisition and retention training was expected to increase the likelihood of perseverative responding; these two “high-difficulty” sessions were conducted at 8 d and 2 weeks after cessation of drug administration (referred to as the “8 d post-exposure assessment” and the “2 week post-exposure assessment,” respectively).

For the purposes of the current study, changes in gray matter were compared to the change in reversal learning performance between the baseline assessment and the 5 d post-exposure assessment, as these assessments used the same performance criterion. The number of trials required to reach criterion in reversal phase of the baseline assessment was subtracted from the number of trials required to reach criterion in the reversal phase of the 5 d post-exposure assessment to calculate a behavioral difference score.

MRI scanning procedures: Each subject underwent structural magnetic resonance (MR) imaging twice: 2-3 weeks prior to initiating the dosing regimen (referred to as the ‘Baseline’ scan) and 3½ weeks

after completion of the dosing regimen (referred to as the 'Post-Dosing Regimen' scan). On scan days, monkeys received an intramuscular injection of ketamine hydrochloride (10 mg/kg) and atropine sulfate (0.01 mg/kg). Once the monkey was sedated, an endotracheal tube was placed to provide inhalation of 2-3% isoflurane gas (in 100% O₂) for the duration of the scan (approximately 1 hr). Nine T1-weighted volumes with three-dimensional, magnetization-prepared, rapid-acquisition, gradient-echo (MPRAGE) images were acquired (TR=1900 ms TE=4.38 ms, FOV=96 mm, flip angle 15 degrees, voxel size 0.5 mm, 248 slices, slice thickness 0.5 mm) using a 1.5-T Siemens Sonata scanner and an 8-channel, high-resolution, knee-array coil (Invivo Corporation).

PET scanning procedures: Three PET scans were collected, as previously described (Groman et al., 2012): (1) ~2 weeks prior to initiating the dosing regimen (referred to as the "baseline scan"), (2) ~2 weeks after the last drug administration to examine the immediate effects of methamphetamine (referred to as "2-week post exposure scan") and (3) ~7 weeks after the last drug administration to examine the stability of neural changes (referred to as the "7-week post exposure scan"). PET scanning was completed using [¹¹C]WIN-35428 and [¹⁸F]fallypride as radioligands for assessing DAT and D₂-like receptor availability, respectively. For the current study, D₂-like and DAT availability measurements collected at the 2-week post-exposure scan were used, as these measurements were closest in time to when the second MR image was collected. Difference scores were calculated by subtracting the availability measurements obtained at the 2-week post-exposure scan from the baseline scan.

Data processing

MR image pre-processing: The nine MPRAGE sequences were motion-corrected using Statistical Parametric Mapping 5 (SPM; Institute of Neurology, University College London, London, England) and averaged. The images were aligned such that the anterior and posterior commissures were in the same plane, field-bias corrected and manually skull-stripped.

Developing tissue class priors for MR image segmentation in vervet monkey: Tissue class priors, which indicate the likelihood of finding a given tissue class at a given location, were created by manually defining and smoothing (3 mm FWHM Gaussian kernel) gray matter, white matter, and cerebrospinal fluid on a vervet monkey template. Ten vervet monkey scans, collected for a different study (Fears et al., 2009), were segmented using SPM5, with the following changes to the default parameters: human tissue classification priors were replaced with the maps of tissue classification created from the vervet template, no affine regularization was conducted, and the sampling distance was changed from 3 mm to 1 mm. The resulting segmentations were averaged to create probability maps of gray matter, white matter and CSF.

Processing of MR images: Baseline and post-dosing regimen MR images for each subject were moved into a position located halfway between the two images, using Advanced Normalization Tools (Avants et al., 2011), and the baseline and post-dosing scans were averaged to create a mean image. The mean, baseline, and post-dosing regimen MR images were then segmented into gray matter, white matter and CSF, using SPM5. The following segmentation parameters were changed from the default settings: human priors were replaced with the vervet monkey priors, no affine regularization was conducted, and the sampling distance was changed from 3 mm to 1 mm. Diffeomorphic Anatomical Registration using Exponential Lie Algebra (DARTEL) was then used to register each subject's mean gray-matter image to template space. Baseline and post-dosing regimen gray-matter images were moved to template space using the nonlinear deformation parameters generated by the registration of each subject's mean gray-matter image to template space (summarized in Figure 1). In the resulting gray-matter images, intensity at each voxel represents the proportion of gray matter with a region.

Reconstruction and processing of PET images: PET images were reconstructed as previously described (Groman et al., 2012). Activity was extracted from each subjects' PET image using previously defined regions of interest and imported into the PMOD kinetic analysis program (PMOD v3.15). Time-

activity curves were fit using the Multilinear Reference Tissue Model (Ichise et al., 2003), using the activity from the cerebellum as the reference. k_2' , the rate constant of tracer transfer from the reference region (cerebellum) to plasma, was extracted from the high-activity areas of the caudate nucleus and putamen and averaged together. The time-activity curves were then refit with MRTM2 using the average fixed k_2' value applied to all brain regions.

Statistical analysis

In order to determine whether exposure to methamphetamine had a different effect on gray matter than saline treatment, difference images were created by subtracting the intensity of gray-matter images obtained from the post-dosing regimen scan from that of the baseline scan. These difference images were then smoothed using a 5 mm FWHM Gaussian function (McLaren et al., 2010). A general linear model (GLM) was created to statistically test the following: 1) whether the difference in gray matter within saline-exposed monkeys was greater than that in methamphetamine-exposed monkeys, 2) whether the difference in gray matter within methamphetamine-exposed monkeys was greater than that in saline-exposed monkeys 3) whether the difference in gray matter between the baseline and post-dosing regimen was statistically significant in saline-exposed monkeys and 4) whether the difference in gray matter between baseline and post-dosing regimen was statistically significant in methamphetamine-exposed monkeys. The GLM was then implemented in FSL RANDOMISE v2.1 tool (Permutation-based nonparametric inference, Oxford University, Oxford UK) with a variance smoothing of 5 mm (FWHM Gaussian). Threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009) was used to detect significant clusters of change; this method provides the ability to perform cluster-based inference without the need to specify an arbitrary cluster-forming threshold, as is necessary when using Gaussian random field theory. For each analysis, 10,000 randomization runs were performed. Statistical maps were thresholded at $p < 0.05$ and corrected for the search volume contained in the following

regions of interest, selected based on evidence that gray-matter volume or density in methamphetamine-dependent individuals is altered in these regions (Thompson et al., 2004; Chang et al., 2005; Jernigan et al., 2005; Morales et al., 2012): orbitofrontal cortex, ventral medial prefrontal cortex, cingulate cortex, insula, caudate, putamen and ventral striatum.

To determine the dependency between changes in gray matter and changes in the number of trials required to reach criterion in the reversal phase in the methamphetamine-exposed monkeys, the difference in the number of trials required to reach criterion in the reversal phase before and after the dosing regimen was regressed against the difference in gray matter using RANDOMISE with variance smoothing of 5 mm (FWHM) and, based on the results of the analysis described above, confined to the search volume of the bilateral putamen. Ten thousand randomization runs were performed, and TFCE was used to detect significant correlation clusters. Statistical maps were thresholded at $p < 0.05$ and corrected for the entire search volume of the putamen.

The relationship between changes in gray matter and changes in D₂-like receptor and DAT availability were examined by regressing the difference in the availability of these dopaminergic markers within the right putamen (2-week post-exposure scan - baseline) against the difference in gray matter using RANDOMISE with variance-smoothing of 5 mm (FWHM) and, based on the results of analysis described above, confined to the search volume of the bilateral putamen. Ten thousand randomization runs were performed, and TFCE used to detect significant correlation clusters. Statistical maps were thresholded at $p < 0.05$ and corrected for the entire search volume of the putamen. Effect size maps (Cohen's *d*) were created by taking the square root of the mean difference in intensity of the gray-matter images in methamphetamine-exposed monkeys to that of saline-exposed monkeys divided by the pooled standard deviation in the difference values.

Correlation coefficients, obtained from Pearson's product-moment correlations, were compared between data from methamphetamine- and saline-exposed monkeys using Fisher's *r*-to-*z* transform.

Results

Brain morphometry: Voxel-wise analysis examining whether methamphetamine produced changes in gray matter that differed from changes in the control condition detected a significant cluster, located within the caudal portion of the right putamen (Figure 2A). A similar cluster was observed in the left putamen; however, it did not survive corrections for multiple comparisons. The simple-effects contrasts examining the difference in gray matter in saline or methamphetamine monkeys indicated that the interaction was due to a significant change in gray matter within the right putamen of methamphetamine-exposed monkeys (Figure 2B), as no significant clusters were detected in the saline-exposed monkeys. Values extracted from the significant interaction cluster revealed that, on average, gray matter increased in methamphetamine-exposed monkeys by 5.2% (+/-1.2% SEM), while gray matter decreased in saline-exposed monkeys by 0.8% (+/- 1.5%; SEM). The overlap between the significant interaction cluster and simple-effect cluster are plotted in Figure 2C, and the raw gray-matter intensity values are plotted in Figure 2D. Effect size (Cohen's d) maps, comparing changes in gray matter between methamphetamine- and saline-exposed monkeys within the regions of interest (Fig. 3), allow for qualitative comparison of differences between groups that did not reach the prescribed statistical threshold.

Acquisition, retention and reversal performance: As previously described (Groman, Lee et al., 2012), analysis of behavioral performance of monkeys before, during and after the dosing regimen revealed no significant differences between the groups for the number of trials required to reach criterion in the acquisition phase or retention phase. However, the number of trials required to reach criterion in the reversal phase significantly diverge between the groups across the behavioral assessments (group by discrimination session: $p < 0.001$). Post-hoc analyses confirmed that this was due to an significant increase in the number of trials required to reach criterion in the methamphetamine-exposed monkeys

between baseline and the three-week assessment ($p < 0.001$); however, no other pairwise comparisons were significant (all p 's > 0.81).

Correlation of changes in inhibitory control with changes in gray matter morphometry: To determine whether the change in putamen gray matter detected in the methamphetamine-exposed monkeys was behaviorally relevant, we tested for a correlation between the change in the number of trials required to reach criterion in the reversal phase after methamphetamine exposure and the difference in gray matter within the putamen. The voxel-wise analysis of this regression revealed a significant cluster located within the caudal portion of the right putamen (Figure 4A), overlapping with the time-by-dosing regimen cluster detected above (Figure 4B). The change in gray matter was positively correlated with the change in reversal-learning performance, such that the increased gray matter was associated with a greater number of trials needed to reach criterion in the reversal phase in methamphetamine-exposed monkeys (Figure 4D). The relationship was not statistically significant in saline-exposed monkeys (Figure 4C).

Covariation of dopaminergic markers with gray-matter morphometry: Previously, we demonstrated that exposure to an escalating dose of methamphetamine significantly reduced D_2 -like receptor and DAT within the striatum of the animals tested here (Groman et al., 2012). To determine whether changes in markers of the dopamine system were correlated with changes in gray matter, the change in these dopaminergic markers within the right putamen before and after the dosing regimen was regressed against changes in gray matter within the putamen. The voxel-wise analysis revealed a significant cluster located within the caudal portion of the right putamen for the change in D_2 -like receptor availability (Figure 5A) as well as the change in DAT availability (Figure 5B). The correlation coefficients did not differ between saline- or methamphetamine-exposed monkeys for D_2 -like receptor availability (Figure 5C) or for DAT availability (Figure 5D).

Discussion

These data provide the first evidence that exposure to a prolonged, escalating dosing regimen of methamphetamine designed to mimic aspects of human use patterns produces long-lasting alterations in the structural integrity of the striatum that can be measured *in vivo* with sMRI, and suggest that the structural abnormalities previously detected in methamphetamine-dependent humans are, at least in part, a consequence of drug use. The degree of change in gray matter within the right putamen correlated with change in reversal-learning performance, providing evidence that the structural alterations have a functional impact on inhibitory control, a process that may be central to addiction. Finally, changes in dopaminergic markers within the putamen were correlated with the change in gray matter, suggesting that the methamphetamine-induced biochemical alterations may be related to the structural change.

Escalation of methamphetamine administration results in increased gray matter in right putamen

Previous studies have indicated that gray-matter volume within the putamen is greater in methamphetamine-dependent humans compared to control subjects (Chang et al., 2005; Jernigan et al., 2005; Churchwell et al., 2012). Although these morphometric differences have long been presumed to be an effect of chronic drug use (Chang et al., 2007), recent evidence has suggested that greater putamen gray-matter volume may be present prior to drug use (Ersche et al., 2012), with these structural differences possibly influencing the development of substance abuse and/or dependence. The results of the current study support the former hypothesis, demonstrating that gray matter in the putamen increased following exposure to methamphetamine in a manner similar to the differences detected in human-methamphetamine users. This is not to say that gray-matter volume differences in the putamen are not both a cause and consequence of stimulant dependence.

Unlike previous studies that have detected gray-matter abnormalities in the prefrontal cortex of methamphetamine dependent humans (Thompson et al., 2004; Morales et al., 2012), prefrontal gray matter was not significantly different in monkeys exposed to methamphetamine than in monkeys exposed to saline. The discrepancy between the human studies and the current study could reflect a variety of factors. One possibility is that changes in prefrontal gray matter produced by 31 days of methamphetamine exposure may be on a smaller scale than that those in putamen, requiring a larger sample size than that used here to detect the change (as evidenced by the effect size maps presented in Figure 3). Changes in prefrontal cortical structure may occur at a slower rate than those in the putamen, emerging only after extensive periods of drug use (several years). Supporting this hypothesis is evidence that prefrontal gray-matter volume/density in heroin- and cannabis-dependent individuals is negatively correlated with duration of drug use (Yuan et al., 2009; Stone et al., 2012). It is also possible that some of the abnormalities in prefrontal cortex detected in methamphetamine-dependent individuals are attributable to factors other than methamphetamine exposure. For example, a large proportion of methamphetamine-dependent individuals smoke tobacco cigarettes, and some abnormalities in prefrontal gray-matter volume observed in methamphetamine-dependent individuals may be attributable to cigarette smoking (Morales et al., 2012). Finally, given that prefrontal gray-matter volume is smaller in drug-naive individuals who are at greater than normal risk for developing alcoholism (Benegal et al., 2007), it is also possible that the differences in prefrontal gray-matter volume observed in methamphetamine abusers predate drug use may reflect an increased vulnerability to developing methamphetamine dependence.

Potential mechanisms for the methamphetamine-induced increase in putamen gray matter

Although several studies have indicated that gray matter in the striatum is altered in methamphetamine-dependent individuals (Chang et al., 2005; Jernigan et al., 2005; Churchwell et al.,

2012; Ersche et al., 2012; Morales et al., 2012), the mechanism by which these alterations occur is unknown. Greater gray-matter volume/density in the putamen may be indicative of increased neuronal density (Tulloch et al., 2011), alterations in the morphology of the existing neurons (e.g., increased spine density) (Jedynak et al., 2007), or, as had been previously proposed, an inflammatory response to methamphetamine exposure (Kousik et al., 2012). In animals, administration of methamphetamine increases activation of astroglia, microglia and other markers of inflammation that have been proposed to mediate the neurotoxic effects of methamphetamine on the dopamine system (Asanuma et al., 2003), and administration of drugs that prevent microglial activation prevents the methamphetamine-induced dopamine alterations (Thomas and Kuhn, 2005). Measurements of microglial activation in methamphetamine-dependent people suggest that reactive gliosis can persist for at least two years following the initiation of abstinence from methamphetamine (Sekine et al., 2008). The changes in gray matter detected in the current study were still present 3 weeks after the final dose of methamphetamine was administered and were correlated with changes in measures of D₂-like and DAT availability, suggesting that changes in putamen morphometry are long-lasting and co-occur with changes in the putamen dopamine system, possibly arising from inflammation.

The increase in gray matter observed here was greater in the right putamen, with a similar, nonsignificant trend observed in the left putamen; however, nonsignificant *decreases* in gray matter were observed in the caudate nucleus (see effect size map, Figure 3). An emerging literature suggest that exposure to methamphetamine has differential effects in striatal subregions. Repeated exposure to methamphetamine increases spine density on medium spiny neurons in the dorsolateral striatum, but decreases spine density in the immediately adjacent dorsomedial striatum (Jedynak et al., 2007), the rodent striatal subregions believed to be orthologous to the primate putamen and caudate, respectively. Methamphetamine exposure also results in alterations in the vasculature of the dorsal but not ventral striatum (Kousik et al., 2011), possibly producing a spatially restricted hypoxic environment and

inflammatory response. Data from human studies suggest that stimulant-dependent individuals have smaller gray-matter volume in the caudate than stimulant-naive individuals (Morales et al., 2012; Moreno-Lopez et al., 2012), in contrast to findings indicating greater volume in the putamen. Taken together, this evidence suggests that methamphetamine exposure differentially alters regions within the striatum, which may differentially alter the behavioral effects of methamphetamine by simultaneously altering the neural circuits that underlie goal-directed (dorsomedial striatum) and habitual (dorsolateral striatum) behaviors. However, additional studies with larger samples are needed.

Correlation of methamphetamine-induced changes in gray matter with changes in inhibitory control

Emerging evidence indicates that the ability to exert inhibitory control over behaviors relies on several regions within the corticostriatal circuit (Castane et al., 2010). Lesions to the orbitofrontal cortex and dorsal striatum produce similar impairments in reversal-learning performance, indicating that the ability to modify behaviors adaptively, upon a change in contingencies, depends upon the coordinated activity of nuclei within the corticostriatal circuit. In the current study, the methamphetamine-induced increases in gray matter in the putamen were positively correlated with the change in reversal-learning performance, demonstrating that the morphometric changes had a detrimental impact on a behavioral process that is altered in human methamphetamine users (Ghahremani et al., 2011).

Conversely, striatal gray-matter volume in human methamphetamine-dependent subjects is negatively correlated with measures of cognitive function (Chang et al., 2005; Jan et al., 2012). The discrepancy between the current findings and this observation may reflect temporal differences in the effects of methamphetamine. For example, during the initial phases of drug use, the effect of methamphetamine on the putamen may be the greatest, producing the largest inflammatory response and alterations to the dopamine system. Consistent with this hypothesis, in adolescent methamphetamine abusers, number of lifetime doses of methamphetamine are positively correlated

with gray-matter volume in the putamen (Churchwell et al., 2012). In adults who abuse methamphetamine, however, years of methamphetamine abuse are negatively correlated with gray-matter volume in the putamen (Chang et al., 2005), suggesting that as use of the drug continues, tolerance develops and compensatory mechanisms are recruited as an adaptation to the persistent presence of methamphetamine, which may then impact cognitive processes in a different way than when methamphetamine use initially began.

These data highlight the neural and behavioral variability that exists between individuals in response to methamphetamine. Despite receiving identical doses and cumulative amounts of methamphetamine, the impact of methamphetamine on gray matter was minimal in some individuals (<2%) and great in others (~11%) suggesting that unidentified biological and/or genetic factors may mediate the neural response of individuals to methamphetamine. The current study found that changes in gray matter within the methamphetamine-exposed monkeys correlated with the changes in reversal learning performance, suggesting that individual differences in methamphetamine-induced gray matter changes have a functional and meaningful impact on behaviors that may contribute to the pathophysiology of methamphetamine dependence.

Future directions for understanding the pathophysiology of methamphetamine dependence

These data support the notion that greater putamen gray-matter volume in methamphetamine-dependent than in methamphetamine-naïve individuals is, in part, a consequence of drug use; however, the etiology and reversibility of these methamphetamine-induced changes remains unknown. Future studies in animals, combining sMRI assessments of brain structure with histological measurements have the potential to link gross anatomical changes to changes at the cellular level. In humans, multimodal neuroimaging, incorporating measures of microglial activation (Sekine et al., 2008) may be used to test the hypothesis that changes in gray matter reflect inflammation. Since the current study found that

exposure to methamphetamine produced an changes in morphometry that functionally impacted behaviors previously shown to predict aspects of sobriety in substance-dependent individuals (Aharonovich et al., 2006; Turner et al., 2009), determining the etiology of these morphometric changes may identify novel targets for the treatment of methamphetamine dependence.

Figures

Figure 1: A diagram of the processing stream used in the current study for the structural MR images.

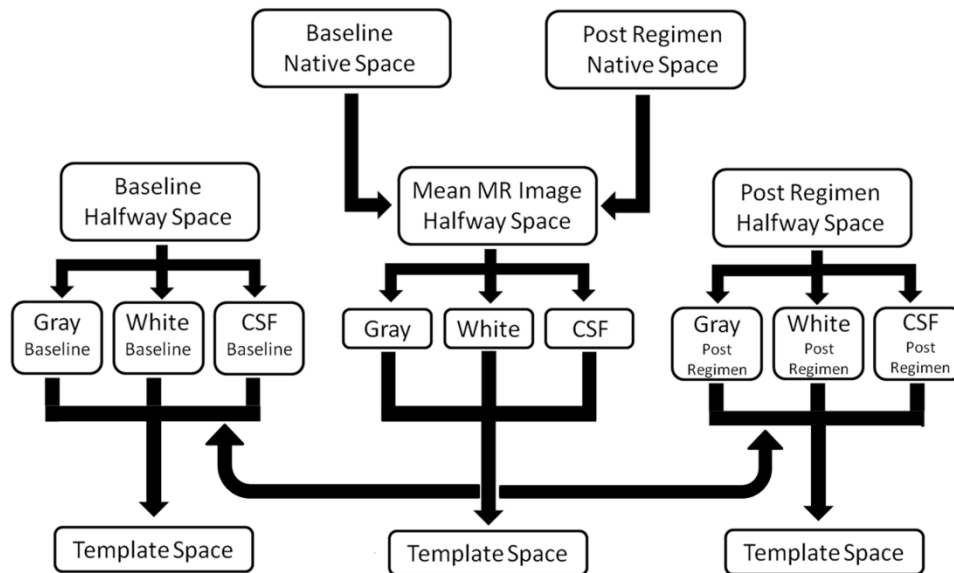


Figure 2: Statistical maps (p values) for the voxel-wise analysis of changes in gray matter with exposure to an escalating dose regimen of saline (SAL; N=7) or methamphetamine (MA; N=7). The significant time-by-drug exposure interaction detected in the right putamen is presented in panel A and the simple effects contrast presented in panel B. The overlap between the interaction cluster and the simple effect cluster is presented in panel C. For illustrative purposes, the intensity of the gray-matter images at the baseline and post-dosing scan in monkeys exposed to saline (closed circles) or methamphetamine (open circles) are presented in panel D.

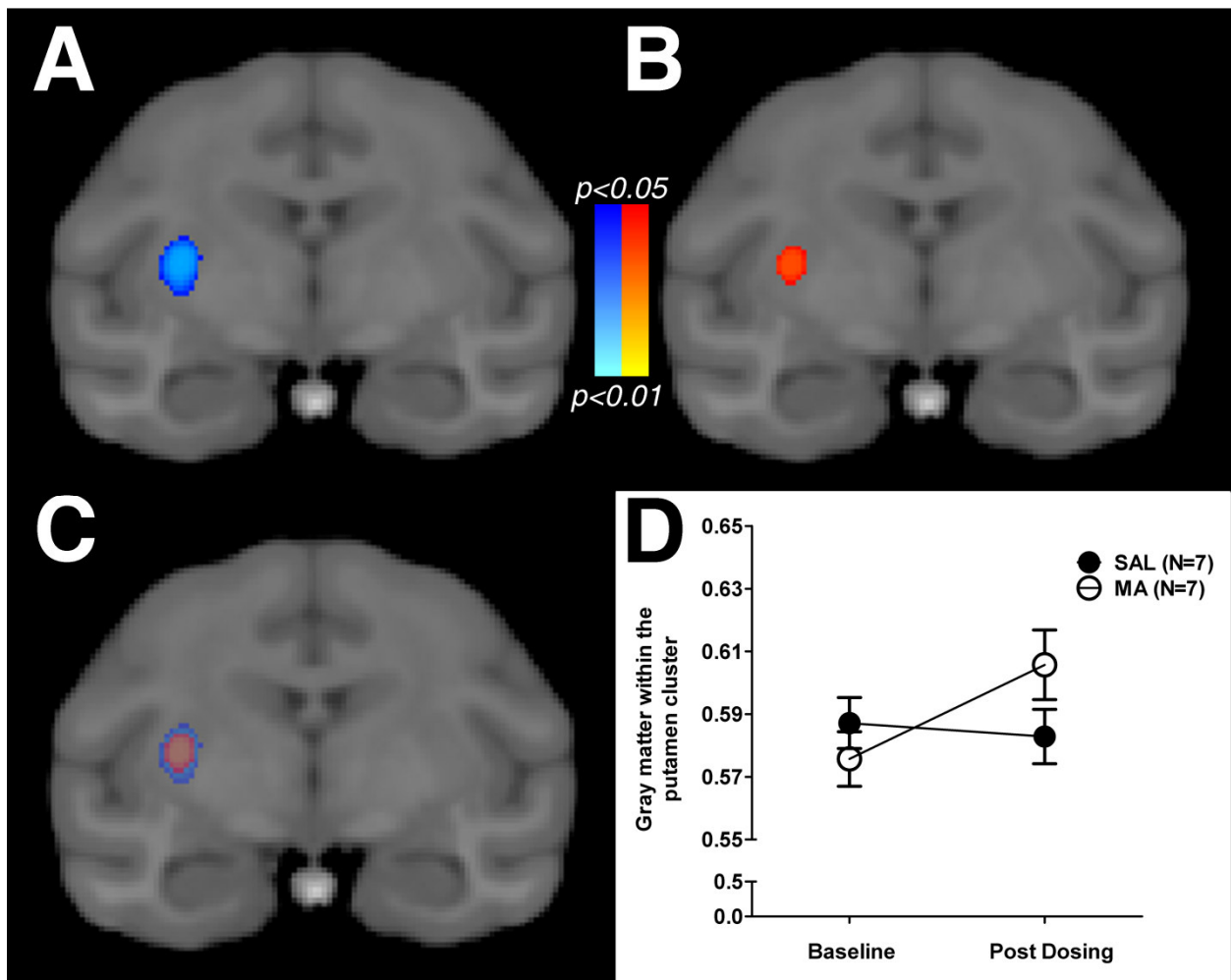


Figure 3: Effect size maps (Cohen's d) comparing the change in gray matter between methamphetamine- and saline-exposed monkeys. Cooler values are indicative of gray matter loss (darker to lighter blue represents lower to greater *negative* effects) in the methamphetamine-exposed monkeys compared to the saline-exposed monkeys, while hotter values are indicative of increases in gray matter (darker to lighter red represents lower to greater *positive* effects) in methamphetamine-exposed monkeys compared to saline-exposed monkeys.

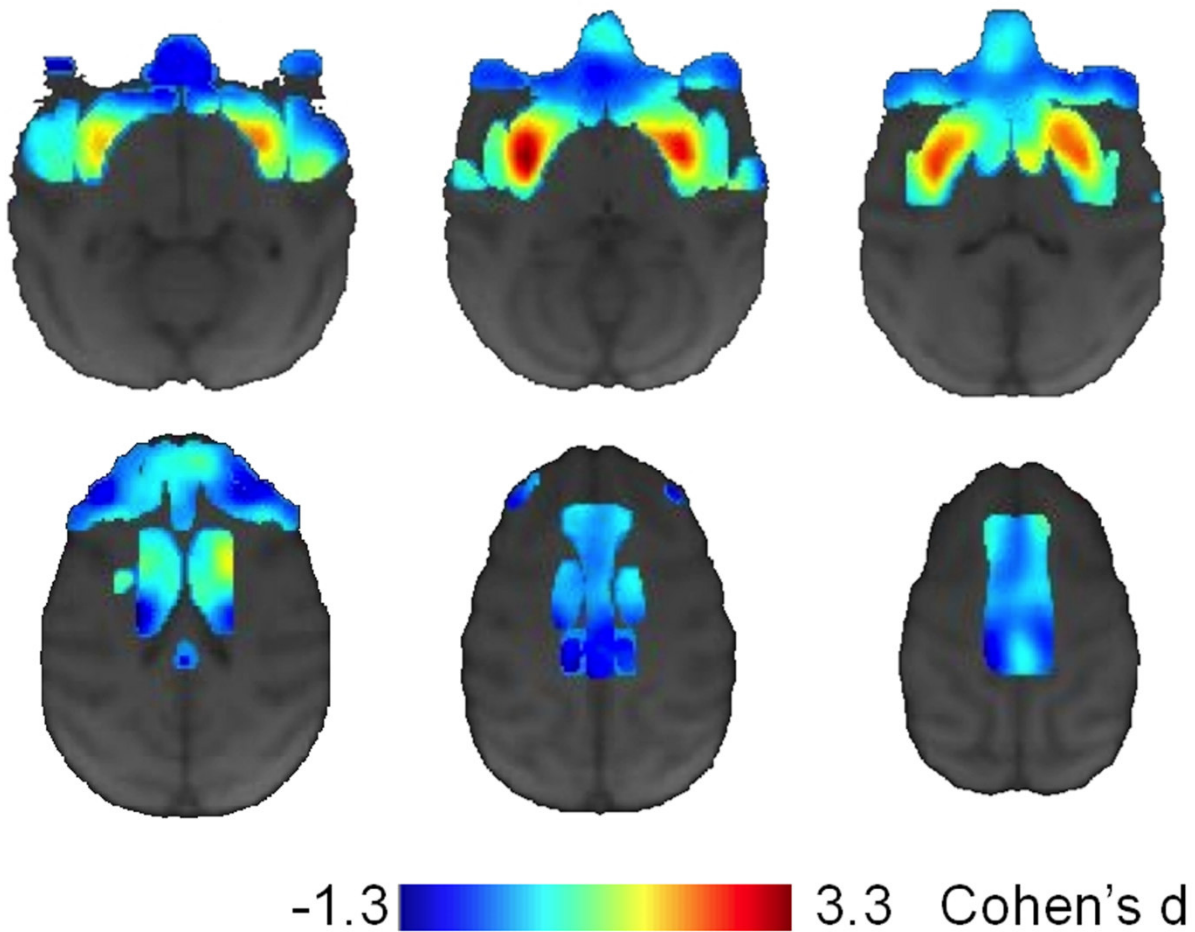


Figure 4: Statistical maps (p values) for the voxel-wise regression of changes in gray matter within the putamen on change in reversal learning performance. The change in the number of trials required to reach criterion in the reversal phase was positively related to the change in gray matter, which is presented in panel A. This cluster overlapped with the time-by-dosing regimen interaction on gray matter as presented in panel B. The relationship was only detected in the methamphetamine-exposed monkeys (panel D), as the change in gray matter did not significantly correlate with the change in reversal learning performance within the saline-exposed monkeys (panel C). Scatter plots are for illustrative purposes only.

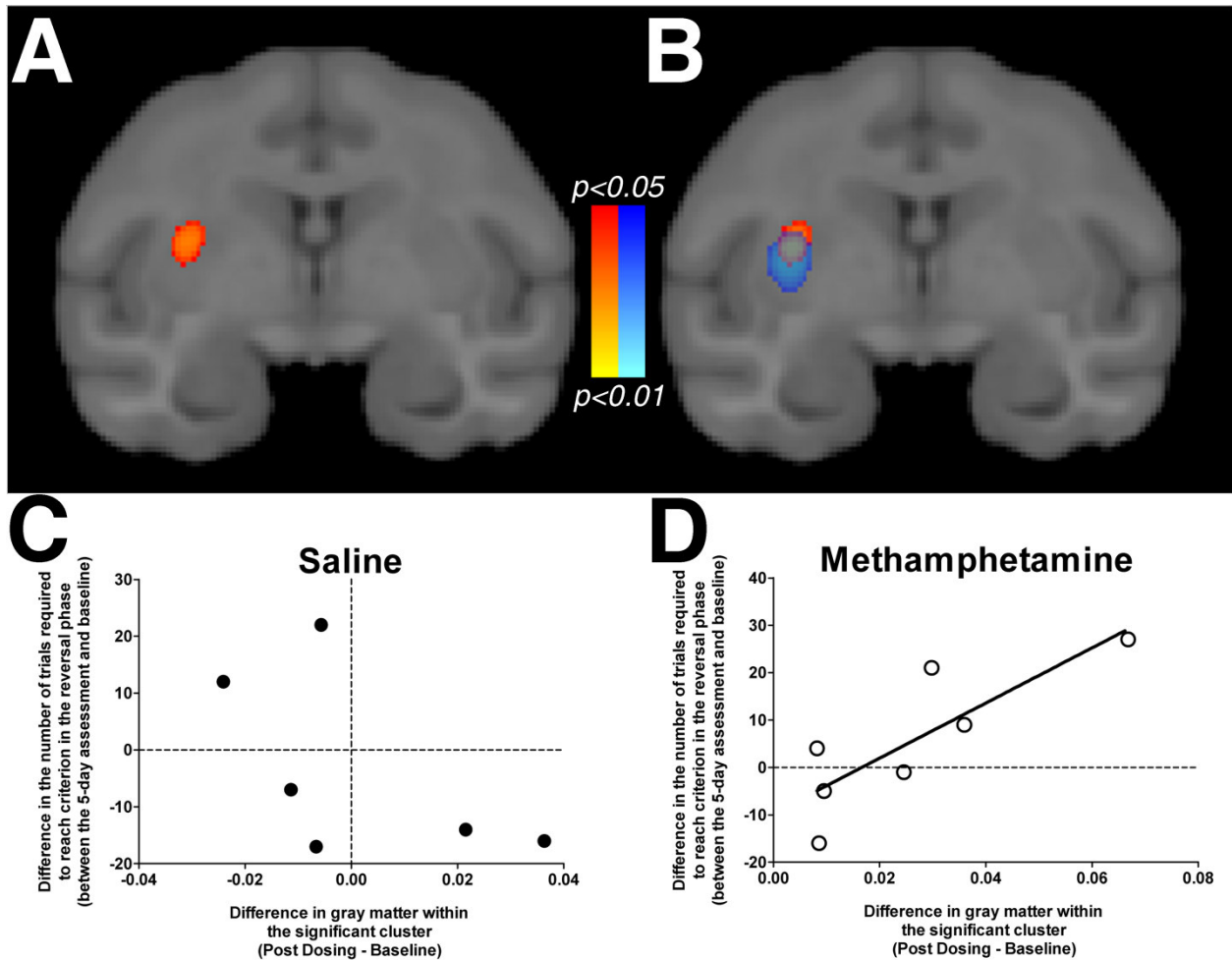
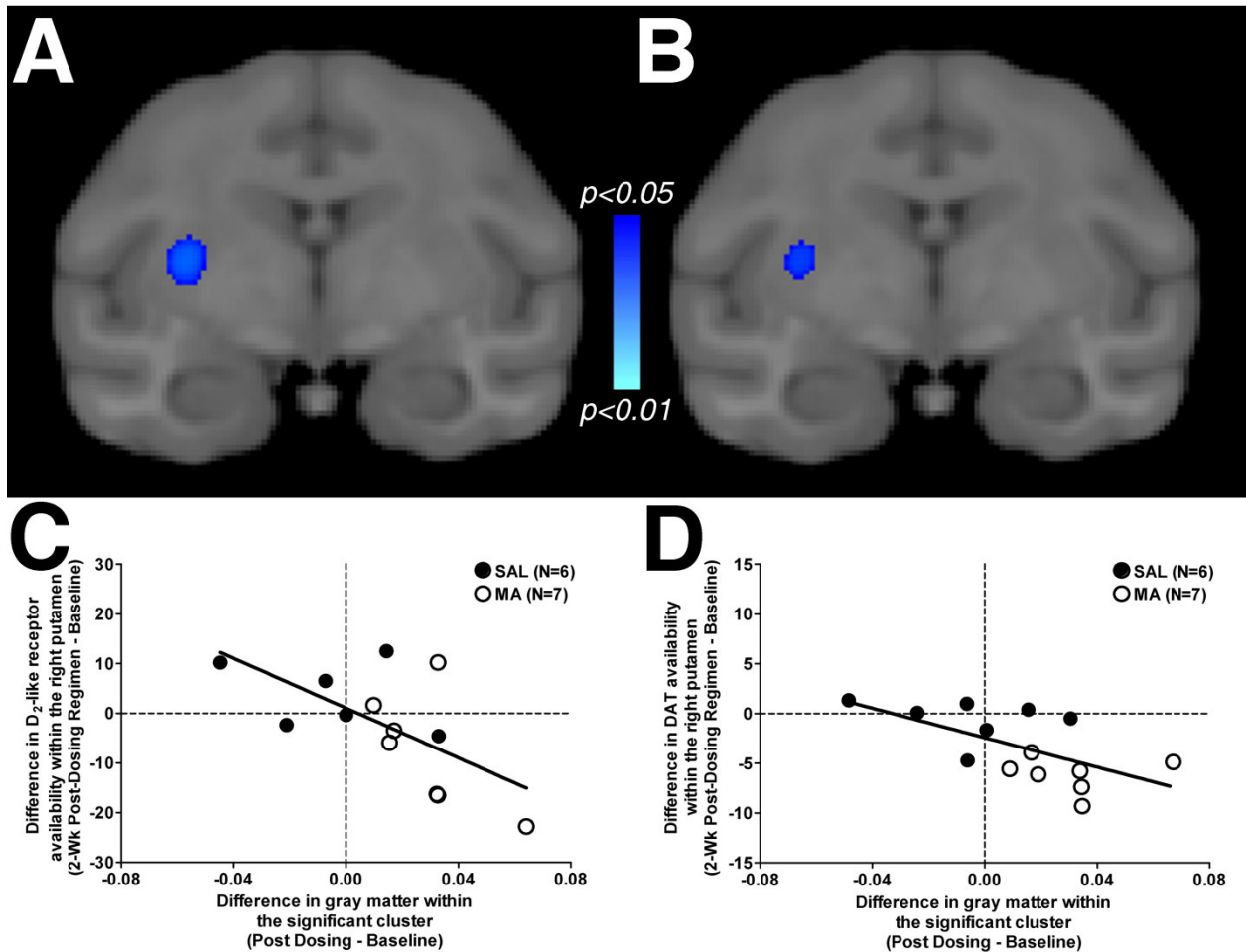


Figure 5: Statistical maps (p values) from the voxel-wise regression of changes in gray matter on changes in D_2 -like receptor availability (panel A) and changes in DAT availability (panel B) within the putamen. Scatter plots demonstrating the relationship between the difference in gray matter (X axis) and the difference in D_2 -like receptor availability (Y axis; panel C) or difference in DAT availability (Y axis; panel D) are presented in both saline-exposed monkeys (closed circles) and methamphetamine-exposed monkeys (open circles).



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