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Dopamine D2 Receptor Priming Enhances Dopaminergic Response to Amphetamine in  
the Nucleus Accumbens: Role of the D1 and D2 Receptors.

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A dissertation

presented to

the faculty of the Department of Anatomy and Cell Biology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy in Biomedical Sciences

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by

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December 2009

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## ABSTRACT

### Dopamine D2 Receptor Priming Enhances Dopaminergic Response to Amphetamine in the Nucleus Accumbens: Role of the D1 and D2 Receptors

by

Kimberly Norris Huggins

In past work, we have shown neonatal quinpirole (dopamine D<sub>2</sub>/D<sub>3</sub> agonist) treatment produces a significant increase in dopamine D<sub>2</sub> receptor sensitivity, a phenomenon known as D<sub>2</sub> receptor priming. Dopamine D<sub>2</sub> receptor priming is common in psychosis. Male and female rats were administered quinpirole (1 mg/kg) or saline from postnatal days 1 -11 and raised to adulthood (P60). As adults, rats were administered d-amphetamine sulfate (1 mg/kg) or saline every other day for 14 days. Approximately 10 min before each amphetamine or saline injection, animals were administered the D1 antagonist SCH 23390 (0.1 mg/kg), the D2 antagonist eticlopride (0.1 mg/kg) or saline. After both injections, rats were placed in a locomotor arena and activity was analyzed for a 10-min period. Results indicated that D<sub>2</sub>-priming enhanced locomotor activation effects to amphetamine in both males and females, with females demonstrating higher levels of behavioral activation. SCH 23390 blocked amphetamine sensitization in both males and females to levels below saline controls, whereas eticlopride was more effective in blocking amphetamine sensitization in males as compared to females, although eticlopride did block elevations of behavioral activation in D2-primed males and females. Seven to 10 days after sensitization, microdialysis was performed and amphetamine produced a five-fold increase in dopamine overflow in the nucleus accumbens compared to non D<sub>2</sub>-primed rats administered amphetamine. Both D1 and D2 antagonism were effective at blocking amphetamine-induced increases in dopamine overflow. These results show that neonatal quinpirole treatment enhances behavioral activation and dopamine overflow, but there appear to be sex differences in the D2 as compared to D1 response to behavioral activation produced by amphetamine.

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## CHAPTER 1

### INTRODUCTION

Substance use disorder comorbidity in mental illness is widespread, impacting up to 50% of patient populations (Siegfried 1998). Schizophrenia is one of the most common dually diagnosed mental illnesses (Cuffel 1996). Dopamine has been implicated in psychosis as being increased in its activity (Schmitt et al. 2009). This increased activity has been debated, but most agree that this neurotransmitter plays a primary role in both motor behavior and drug reinforcement. In our laboratory, we have used a rodent model of increased dopaminergic activity to analyze drug abuse. This model is focused on increasing the sensitivity of dopamine D2-like receptors in the brain, that is accomplished through developmental treatment with a dopamine D2/D3 agonist quinpirole. Previous work using this model has shown a potentiated effect of amphetamine on dopamine overflow within the dorsal striatum (Nowak et al. 2001). This effect is consistent with findings in schizophrenia (Abi Dargham et al. 1996; Laurelle et al. 1996). Thus, these results suggest that psychotics who use amphetamine may demonstrate a sensitized dopamine response to amphetamine. If this is the case, then this increased dopamine response may be producing a stronger euphoric reaction to stimulant drugs in this population, and this may be the basis for the increased usage of these drugs in this population.

This study uses these past findings as a springboard to analyze the effects of subchronic amphetamine treatment on locomotor sensitization and accumbal dopamine as well as to identify sex differences on these measures. In addition, the roles of dopamine D1 and D2 dopamine receptors in sensitization and the dopaminergic

response to amphetamine are studied. These findings may lead to identifying receptor mechanisms of this effect, be the first study to analyze the roles of D1 and D2 receptors in both males and females in this phenomenon, that may lead to further understanding the significant health problem of substance abuse in psychosis.

#### Dopamine D2 Receptor Priming Model

Kostrzewska and colleagues have shown that quinpirole, a dopamine D2/D3 receptor agonist, administered during neonatal development results in increases in dopamine D2 receptor sensitivity throughout the animal's lifetime. This is a phenomenon that has been referred to as priming of the D2 receptor. Previous work has shown that quinpirole treatment from postnatal days 1-11, 1 -21, or 21 -35 results in an increase of dopamine D2 receptor sensitivity that persists throughout the animal's lifetime (Kostrzewska et al. 1990, 1991, 1993a, 1993b, 1995). This change is independent of a change in D2 receptor number. In recent work, we have shown that the neonatal quinpirole treatment model has produced several behavioral changes that are consistent with findings in schizophrenics.

First, as mentioned above, acute amphetamine administration to adult rats neonatally treated with quinpirole produced a robust increase in dopamine overflow in the striatum greater than an increase seen by amphetamine administration alone (Nowak et al. 2001). This is consistent with findings from clinical studies using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and single positron emission computer tomography (SPECT) in schizophrenic patients that have shown a pronounced increase of amphetamine-induced dopamine release in the dorsal striatum as well as a decrease of amphetamine-induced dopamine depletion

in drug-naive schizophrenic patients, indicating a regulatory problem with dopaminergic neurotransmission (Lanzenberger and Kasper 2005, Laruelle, et al. 2005).

Second, a hallmark of psychosis is a deficit in sensorimotor gating, that is most commonly analyzed using the behavioral paradigm prepulse inhibition (PPI). PPI is a psychophysiological index of sensorimotor gating. One of the more consistent behavioral findings in schizophrenics is that PPI is impaired, that has also been suggested to be related to cognitive impairments known to exist in this population (Geyer 1998). In medicated schizophrenia patients, greater PPI impairment during the attended prepulse has been associated with heightened positive symptoms related to the disturbance (Dawson et al. 2000). We have shown that in previous work that adult rats that were neonatally treated with quinpirole demonstrated deficits in sensorimotor gating as tested by PPI (Maple 2005).

Third, neonatal quinpirole treatments have been shown to produce long-term cognitive impairment in both adolescents and adulthood (Brown et al. 2002; 2004; 2005). Cognitive impairment is present in schizophrenia and has been suggested to be the 'core' of behavioral deficits present in the disorder (Elvevag and Goldman 2000). This suggestion follows the discovery that 94% of patients with schizophrenia have neurocognitive deficits in the areas of memory, attention, and executive function and may help predict the functional outcome in schizophrenia (Waldo et al. 1994; Green 1998). Often, these deficits constitute one of the key features in a schizophrenia diagnosis (Peuskens et al. 2005).

Fourth, possibly the most important neurophysiological finding consistent with psychosis is that neonatal quinpirole treatment produces a significant decrease in

RGS9 expression in the nucleus accumbens, striatum, and frontal cortex of adult rats (Maple et al. 2007). RGS9 is a regulator of G-protein signaling at the D2 receptor, and when active RGS9 increases the rate of termination of events at the D2 receptor, ultimately resulting in a lower activity in the D2 receptor (Rahman et al. 2003). Thus, a decrease in RGS9 expression is consistent with an increased signaling at the D2 receptor. Importantly, significant decreases in RGS9 have been shown in these same brain areas post-mortem in human psychotic patients (Seeman et al. 2007).

Fifth, subchronic treatment with the atypical antipsychotic olanzapine has been shown to alleviate cognitive deficits and long-term priming of the D2 receptor produced by neonatal quinpirole treatment (Thacker et al. 2006). Olanzapine is in the atypical class of antipsychotics and blocks the D2 receptor with some affinity as well at the 5-HT2 receptor. Olanzapine is one of the most often prescribed antipsychotics for schizophrenia. Our laboratory has shown that sub-chronic olanzapine treatment given twice daily for 1 month in adulthood modestly alleviated deficits in Morris water maze performance produced by neonatal quinpirole treatment (Thacker et al. 2006). Importantly, this treatment also alleviated the significant increase in yawning produced in rats neonatally treated with quinpirole to control levels after a 7-day washout. Yawning behavior is a dopamine D<sub>2</sub>/D<sub>3</sub> receptor mediated event (Cooper, et al. 1989; Collins, et al. 2005). These data demonstrate that not only is D<sub>2</sub>-priming likely primarily responsible for these behavioral effects, but that antipsychotic treatments are effective in alleviating cognitive impairment, that is also consistent with clinical findings in schizophrenics (Gurpegui et al. 2007).

Sixth, another neurochemical consistency in this model is that neonatal quinpirole treatments have been shown to result in neurotrophic factor abnormalities comparable to observations in human schizophrenic patients. Studies from this lab have shown a significant decrease in nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) protein in the hippocampus of D2-primed animals as compared to control treated animals. Further, significant decreases in hippocampal NGF and BDNF protein produced by neonatal quinpirole treatment were reversed by olanzapine, and brain tissue was not taken until after an 8-day washout. Interestingly, sub-chronic olanzapine treatment failed to alleviate significant decreases of genetic expression of NGF or BDNF in the hippocampus or frontal cortex as measured through real-time PCR (RT-PCR; Brown et al. 2008), suggesting that the positive effects of olanzapine on neurotrophic factors in this model may be focused on changes in protein and not genetic expression. This is consistent with findings that have shown non-medicated schizophrenic patients have an overall decrease in NGF and BDNF expression, hypothetically involved in neurodevelopmental abnormalities in the hippocampus and frontal cortex known to be present in schizophrenia (Aloe et al. 2001, Parikh et al. 2003).

These six consistencies demonstrate the validity of the model relative to psychosis. However, this model should be used with some caution. Although an increase in dopamine D2 receptor sensitivity is a hallmark of psychosis, it is not the only change responsible for the array of behavioral abnormalities known to exist within this disorder. For example, it is also well-known that there is hypoactivity at the glutamatergic N-methyl-D-aspartate (NMDA) receptor that is important for many of the

behavioral and neurochemical abnormalities mentioned above (Labrie and Roder, 2009), and may be important in the increased substance abuse within this population. Further, there are also important changes in the serotonergic system that also may play a role in substance abuse in psychosis as well, and we do not yet know whether these neurotransmitter systems are also disrupted in the same manner within this model. Regardless, increases in D2 receptor sensitivity are a hallmark of psychosis, and we have shown several important consistencies between this model and the human condition.

### Psychosis

Psychotic symptoms can occur in several behavioral disorders including schizophrenia, bipolar disorder, obsessive-compulsive disorder, degenerative brain disease, as well as following the abuse of steroids. While these diseases exhibit unique characteristics, one common biomarker is a putative increase in the high-affinity states of dopamine D2 receptors in the central nervous system as a property of psychosis (CNS; Seeman et al. 2005). The D2 dopamine receptor, originally denoted as the antipsychotic receptor, has been examined for changes in density following various treatments, most commonly denervation of the neostriatum and long-term administration of antipsychotics (Seeman et al. 1980; Schwarting and Huston 1996,). Most importantly, increases in D2 receptor sensitivity are the basis of the dopamine hypothesis of schizophrenia, as all antipsychotic drugs block the D2 receptor.

### Dopamine Hypothesis of Schizophrenia

The first formulation of the dopamine (DA) hypothesis resulted from early observations that activation of the dopamine system by psychostimulants produced a behavioral phenomenon that is consistent with psychosis, and that dopamine antagonists relieved this psychotic state (Rossum 1966). These observations led to the hypothesis that hyperactivity of DA transmission was responsible for the disorder. The classic dopamine hypothesis was further bolstered from the correlation between clinical doses of antipsychotic drugs and their potency at blocking DA D2 receptors as well as the psychotogenic effects of DA-enhancing drugs (Creese et al. 1976; Lieberman et al. 1987; Seeman 1987).

The current hypothesis suggests that an imbalance in DA with hyperactive subcortical mesolimbic projections results in hyperstimulation of D2 receptors. This increased sensitivity of D2 receptor causes positive symptoms and hypoactive mesocortical DA projections to the prefrontal cortex (PFC) results in hypostimulation of D1 receptors, that ultimately results in negative symptoms and cognitive impairment (Tzschentke 2001). Weinberger has suggested that the two areas of DA imbalance may be related because a deficiency in mesocortical DA function may translate into disinhibition of mesolimbic DA activity (1987).

Dopamine has been a major focus of the research involving the mechanisms of schizophrenia. Much of the literature suggests a combined impact of glutamatergic and dopaminergic systems as the mechanism underlying psychosis, and some studies have focused on dysfunction in the glutamate system. While each system has shown results lending merit towards an understanding of the disease, it remains difficult to

reconcile the hypoglutamatergic and hyperdopaminergic hypotheses. It is most plausible that the heterogenous symptomology of the disease arise from different neurotransmitter systems. The dopamine hypothesis is no longer the only accepted theory as the basis of schizophrenia but still holds validity in the study of many aspects of the disease.

### Modeling Psychosis

#### Using Animal Models and Their Importance

A recent focus of neuroscience research and of the National Institutes of Health has been on the formation of models of human diseases (NIH Roadmap, 2005). Overall, animal models add value in basic sciences research by allowing investigators to elucidate aspects of complex disease or mechanistic pathways. Animal models are limited by the inability to study the long-term progression of a disease and the lack of direct homology between animal systems and the human system (Woodruff and Baisden 1994). However, animal models may be useful for development and discovery of new and more effective treatments for neurological disorders (Woodruff & Baisden 1994). In many cases, animal models of neurological disease and dysfunction are used to model one aspect of the disorder whether it be behavioral, neurochemical, or neuropathological. This can be extremely useful because it can be informative about the contribution of this behavioral or neurochemical abnormality to the disease or disorder, although the entire disorder is not modeled in the animal. McKinney and Brunney (1969) proposed three criteria for validating an animal model of similar inducing conditions, similar behavioral states, and common mechanisms in an animal model. The D2-priming model used in this study reaches all three of these criteria, as mentioned above.

McKinney and Brunney (1969) concluded that the higher number of criteria the model achieves means that the model has increased validity. Therefore, the model used here has increased validity relative to the psychotic state, although we have not yet shown whether there are changes in non-dopaminergic systems that are consistent with the disorder. Therefore, to research a clinical condition in humans, an animal model that does not necessarily have all aspects of the disorder can be used for a more thorough examination of the disease.

#### Animal Models of Psychosis

Modeling schizophrenia and/or psychosis poses several difficulties as a result of the heterogeneity of the presentation of symptoms. Animal models are used in studying schizophrenia, psychosis as an aspect of mental illness, drug-induced psychoses, and bipolar disorder that may include a psychotic component. The psychotic component of schizophrenia has garnered much attention adding to the understanding of psychosis in general. Schizophrenia is a severe and chronic mental illness affecting approximately 1% of the population. Symptoms of schizophrenia typically emerge during adolescence or early adulthood (American Psychiatric Association, 2008). Symptoms are usually classified into three categories: positive (hallucinations, delusions, thought disorganization), negative (anhedonia, social withdrawal), and cognitive (deficits in attention and memory) (Carpenter and Buchanan, 1994). Hallucinations can be auditory, visual, tactile, or olfactory in nature. Auditory hallucinations include hearing voices and sounds that are not audible to outside observers (American Psychiatric Association Diagnostic and Statistical Manual 2000). Negative symptoms include a flattened affect or lack of emotion, intonation or facial expression, as well as anhedonia

(absence of pleasure and lack of motivation or ability to follow through with plans).

Clinical diagnoses recognize impaired social relationships, difficulty in maintaining self-care, and impaired social and professional interactions as part of the disorder. Previous research suggests that alterations in several neurotransmitter systems are involved in the pathophysiological process leading to the expression of these symptoms, though no clear etiology of schizophrenia has been shown.

#### Other Rodent Models of Schizophrenia

Several animal models have been used to investigate aspects of schizophrenia. The importance of animal research is evident in the ability to generalize findings using animal subjects to the human health condition. Generalization of schizophrenic research from animal to human is possible through using behavioral tasks with specific task demands, testing parameters, and mechanisms of the behavioral deficits involved in the disorder. There have been several attempts to model schizophrenia using rodent models. All of these models have clinical relevance to the disorder through mimicking neurochemical abnormalities known to be present in schizophrenia. However, it is also important to realize that all of these models have weaknesses that relate to the complexity of the neurochemical aberrations in this disorder.

#### NMDA Receptor Model

A primary abnormality in schizophrenia is related to the glutamatergic system. The glutamate hypothesis suggests that glutamatergic dysfunction is associated with the NMDA receptor. Research has found that a glutamatergic receptor subtype, the N-methyl-D-aspartate (NMDA) receptor, is less responsive in schizophrenics than it is in controls (Tsai and Coyle 2002, Coyle 2006). Based on NMDA hypofunction,

researchers have focused on producing NMDA hypofunction through acute administration of phencyclidine (POP) or ketamine, that are both noncompetitive antagonists of the N-methyl-D-aspartate (NMDA) glutamate receptor. Studies have shown that NMDA receptor hypofunction results in positive and negative symptoms similar to those found in schizophrenia as well as cognitive deficits in normal humans (Snyder 1980, Javitt and Zukin 1991, Tamminga 1998). NMDA receptors are a major subtype of glutamate receptors that mediate slow excitatory postsynaptic potentials (EPSPs) that are considered critical for the expression of behaviors including associative learning, working memory, and attention, functions that are impaired in schizophrenia (Blair et al. 2001). POP has also been shown in rats and humans to closely mimic the positive, negative, and cognitive symptoms observed in schizophrenia (Krystal et al. 1994, Carlsson et al. 2004, Lisman et al. 2008). Neuronal synchronization, that is thought to depend partly on glutamatergic regulation over GABAergic neurons, is impaired in schizophrenia and correlates with perceptual, speech, and other cognitive processes (Spencer et al. 2003, Ford et al. 2007, Lewis et al. 2005, Mohler 2007, Gaspar et al. 2009). Using a novel object recognition paradigm, rats treated with POP were shown to produce robust reductions in time spent with the novel object versus the familiar object, a measure of working memory (Grayson, et al. 2007). The novel object recognition task, that is used to measure working memory, was developed based on the natural propensity of rats to explore novel objects (Ennaceur and Delacour 1988). A reduction in working memory following POP administration helps to establish some validity of the model in terms of investigating cognitive deficits that may exist in schizophrenia. This model would not withstand the criteria for face

validity as many of the symptoms of schizophrenia are subjective in nature and cannot truly be replicated in an animal model. However, glutamatergic manipulations have value as a predictive model as such manipulations induce behavioral or neurochemical changes similar to schizophrenia and are alleviated by similar therapeutic treatments.

### Psychostimulant Model

Acute administration of psychostimulants is known to produce a robust increase in dopamine release, and increased dopaminergic activity as a result of psychostimulant treatment was the original basis of the dopamine hypothesis of schizophrenia (Duncan et al. 1999). Increases in dopamine function, as mentioned above, are known to be common in schizophrenia. In the psychostimulant model, animals are tested while under the influence of the drug to analyze behavioral changes related to robust increases in the dopamine system (Tilson & Rech 1973, Lacriox et al. 2000).

Two behavioral measures used in an effort to model schizophrenia that seem to have clinical relevance are latent inhibition and prepulse inhibition (PPI). Latent inhibition (LI) is a process by which exposure to a stimulus of little or no consequence prevents conditioned associations with that stimulus from being formed. Latent inhibition has been interpreted as a representation of the lack of the ability to ignore irrelevant information, a deficit observed in schizophrenic patients (Solomon et al. 1981, Killcross et al. 1994). Disruption in LI has been demonstrated in schizophrenia (Gray et al. 1992, 1995, Lipp et al. 1992). The operational processes can be viewed as analogous across species (Lubow and Gewritz 1995). Prepulse inhibition (PPI) is a psychophysiological index of sensorimotor gating that has been used with schizophrenic patients. PPI is defined as the reduction of the amplitude of the startle reflex (usually

the eyeblink reflex in humans) when a “prepulse” that is non-startling precedes the startle pulse (Blumenthal 1999). PPI has been found to be impaired in schizophrenic patients. For example, using treatments of amphetamine with and without DA antagonists, it was shown that the medial prefrontal cortex is involved in mediating prepulse inhibition but is not involved in latent inhibition (Lacroix et al. 2000). Thus, cortical changes proposed in schizophrenia may be modeled using PPI measurements but not LI. Although these treatments are helpful in elucidating the effects of short-term increases in DA release, they are unable to recreate the long-term dopaminergic effects seen in schizophrenia. Therefore, the acute administration of a pharmacological substance may be useful as a predictive model but does not meet the constraints for face validity or construct validity.

#### Neonatal Ventral Hippocampal Lesion (NVHL) Model

Likely the most prominently used model of schizophrenia has focused on neonatal damage of the ventral hippocampus in rats (Lipska et al. 1993, Chambers et al. 1996, Wan et al. 1996, Brake et al. 1999, Grecksch et al. 1999). Past results have shown that lateral ventricles are larger and that the hippocampus is smaller in volume in schizophrenics. The main objective of this model is to disrupt hippocampal development that is a hallmark of neuropathology known to be present in schizophrenia (Falkai and Bogerts 1986; Bogerts et al. 1990; Suddath et al. 1990; Eastwood and Harrison 1995; 1998; Weinberger 1999). The disruption of hippocampal development also results in the changes of the cortical and subcortical circuitry of brain areas that make connections to the hippocampal formation. These neonatal lesions are intended to involve regions of the hippocampus that directly project to the prefrontal cortex,

including the ventral hippocampus proper and ventral subiculum that corresponds to the anterior hippocampus in humans (Jay et al. 1989; Carr and Sesack 1996). These lesions produce changes in hippocampal-frontal cortex connectivity consistent with schizophrenia as well as changes in behavioral deficits in latent inhibition, prepulse inhibition, social responses, and cognitive performance (Swerdlow et al. 1996, Lipska et al. 1995, Chambers et al. 1996, Grecksch et al. 1999). Further, these changes do not emerge until after adolescence, consistent with the presentation of schizophrenia in humans (Lipska et al. 1993, Chambers et al. 1996, Becker et al. 1999, Lipska et al. 1995). The lesions result in the clear destruction of ventral portions of the hippocampus including parts of the CA1, CA2, and CA3 regions and portions of the ventral subiculum (Swerdlow et al. 2001). A major weakness of this model involves the blatant neuronal damage created by the lesion. This disconnection of neurons is not exhibited in schizophrenia and the impact of such disruption outside of the targeted effects may be yet unknown.

#### Comorbidity of Substance Abuse

Drug addiction is viewed as a complex neuroadaptive process through that drugs of abuse alter cellular and molecular aspects of neural function to increase or decrease the responsiveness of various behavioral effects through their mediating circuits (White 2002). The dopaminergic system is most directly implicated in the positive reinforcing effects of all drugs of abuse. The ability of addictive drugs to enhance dopamine neurotransmission, primarily in the mesolimbic dopaminergic system, has been documented widely across various classes of abused drugs (Wise and Bozarth 1987, Koob 1992, White 1996, Spanagel and Weiss 1999).

### Substance Abuse in Schizophrenia

Substance abuse in schizophrenics carries consequences that further impact schizophrenic patients. Research has shown that among individuals diagnosed with schizophrenia between 33% and 66% meet the criteria for at least one substance related disorder in their lifetime (Meuser et al. 1992). Schizophrenic patients with concurrent substance abuse experience more frequent hospitalization episodes (Swofford et al. 2000). The interactions of the drugs of abuse with the neuropathology of schizophrenia exacerbate symptoms and interfere with drug treatments (Burnette et al. 1997, Dervaux et al. 2001). Therefore, substance abuse in schizophrenics complicates the proper medication of these patients (Siris et al. 1998).

Two hypotheses have been proposed to explain the increased rate of substance abuse in schizophrenics. The first hypothesis suggests that schizophrenic patients abuse psychostimulant drugs in an attempt to alleviate aspects or symptoms of the disease (Akerele and Levin 2002, Gregg et al. 2007). Studies have suggested that nicotine use may help alleviate sensory gating abnormalities and attentional deficits produced by schizophrenia (Le Due and Mittleman 1995, Adler et al. 1998). The second hypothesis focuses on the use of drugs to relieve negative side effects of the neuroleptic treatment of the disease. Antipsychotic medications have been used in schizophrenia treatment since the early 1950s. While these medications are helpful in allaying positive symptoms, antipsychotic treatment can also lead to disabling extrapyramidal motor symptoms such as parkinsonianism, dystonia, and dyskinesia. Studies have attempted to address whether the use of psychostimulants in schizophrenic patients exacerbates extrapyramidal symptoms or is sought to alleviate those symptoms

(Dixon et al. 1991, Gao et al. 2008, Potvin et al. 2009). Results have shown that patients with a dual diagnosis of schizophrenia and substance abuse exhibit worse extra-pyramidal symptoms than either disorder singly (Duke et al. 1994, Bailey et al. 1998, Potvin et al. 2009). Clinical studies have not been able to determine the order of occurrence in the relationship between substance abuse in schizophrenics and extrapyramidal symptoms. Confounding factors include a varied dosage of antipsychotics by individual, a variety of abused drugs often within a single subject, and the potential symptoms of psychostimulant withdrawal that may mimic some of the extrapyramidal symptoms.

Another mechanism of studying the relationship between substance abuse and schizophrenia is the self-report. Within the self-reporting literature, several reasons for use have been examined: intoxication effects, social reasons, dysphoria relief, and alleviation of psychotic symptoms and to mitigate extrapyramidal symptoms. Between 35% and 95% of subjects reported using drugs or alcohol for the intoxicating effects (Dixon et al. 1991, Addington and Duchak 1997, Baker et al. 2002). Between 8% and 81% of schizophrenic patients cited social reasons as the cause for their drug use (Test et al. 1989, Dixon et al. 1991, Warner et al. 1994). Up to 86% reported using drug or alcohol to relieve dysphoria (Test et al. 1989, Dixon et al. 1991, Warner et al. 1994). In using drugs or alcohol to alleviate or cope with psychotic symptoms, results indicated that up to 42% of subjects responded affirmatively (Addington and Duchak 1997, Dixon et al. 1991, Test et al. 1898). Between 0 and 48% reported using alcohol or drugs to alleviate negative side effects of medication (Test et al. 1989, Dixon et al. 1991, Warner

et al. 1994, Gregg et al. 2007). Overall, there is no clear understanding of the full relationship of the dual diagnosis of schizophrenia and substance abuse.

### Dopaminergic Mechanisms of Amphetamine

Amphetamine is an indirect dopamine agonist known to increase extracellular dopamine in the striatum and nucleus accumbens (Wise 1996). Amphetamine induces increases in dopamine overflow and prevents dopamine reuptake through inhibition of the dopamine transporter. In addition to inhibiting dopamine reuptake, amphetamine causes the release of dopamine from the presynaptic terminals (Seiden et al. 1993).

Amphetamine-induced dopamine release occurs by two mechanisms: calcium-dependent vesicular release and impulse-dependent or transporter-mediated release. Impulse-dependent release is independent and has little calcium dependence that is why often amphetamine is stated to increase dopamine overflow as compared to dopamine release (Hurd and Ungerstedt 1989, Pierce and Kalivas 1997).

Amphetamine can cross the plasma membrane via lipophilic diffusion as well as being a substrate for the dopamine transporter (DAT) (Zaczek et al. 1991a, 1991b). Once inside the neuron amphetamine (AMPH) displaces dopamine from the vesicles into the cytoplasm (Sulzer and Rayport 1990). From the cytoplasm, DA can be released into the extracellular space by the dopamine transporter (Floor and Meng 1996). Studies have shown that both the depletion of dopamine from secretory vesicles and the reversal of DAT-mediated transport are necessary for the effect of amphetamine on overflow in dopaminergic neurons (Jones et al. 1998).

### Behavioral Sensitization

One common behavioral test to analyze the effects of amphetamine on behavioral activation is behavioral locomotor sensitization. Behavioral sensitization is the progressive and long-lasting augmentation of certain behaviors following repetitive stimulant drug treatment (Segal and Mandell 1974, Kalivas and Stewart 1991, Robinson and Berridge 1993). Sensitized behaviors may occur with a shorter latency, higher intensity, or at a lower dose following repeated treatment with stimulant drugs including amphetamine or cocaine (Segal et al. 1980). Behavioral sensitization is a two-step process, development and expression. The development or initiation of amphetamine sensitization involves dopamine D1 and glutamate receptor activation within the ventral tegmentum and substantia nigra (Kalivas and Alesdatter 1993, Wolf et al. 1995, Bijou et al. 1996). Expression is defined as the neuronal alteration arising from the initiating process that mediates the augmented behavioral response and that is mediated by the nucleus accumbens (Wolf 1998). Alterations include an increase in the non-NMDA glutamate AMPA receptor surface expression resulting from dopaminergic modulation in the nucleus accumbens following psychostimulant sensitization (Pierce 1996, Boudreau and Wolf 2005). Measurements of locomotor activity including vertical, horizontal and rotational are typically used in experiments analyzing behavioral sensitization.

Behavioral changes resulting from addictive drugs resemble behavioral responses elicited by natural reward, though the reward of addictive drugs has the power to supplant all other goals, making the drug reward a higher priority to the organism than all other rewards including food. The rewarding properties of addictive drugs putatively require an increased dopaminergic release from midbrain ventral

tegmental area neurons onto nerves in the nucleus accumbens (Wise and Bozarth 1987, Koob and Bloom 1988, Di Chiara 1998). Ventral tegmental area dopamine projections to other forebrain areas such as the prefrontal cortex and the amygdala also play a role in shaping drug-taking behaviors (Everett et al. 1999). Structural similarities in the nucleus accumbens shell and amygdala have led some to postulate the nucleus accumbens shell to be an extension of the amygdala, the region of the brain thought to be responsible for emotional and rewarding behaviors (Everitt et al. 1991, Meredith et al. 2002). Despite the varying targets and chemical composition of the various classes of addictive drugs, they all cause an increase in synaptic dopamine within the nucleus accumbens. In order for addictive drugs to produce reward, they must utilize dopamine in a similar manner (Kelley and Berridge 2002). It has been hypothesized that addictive drugs have competitive advantages over natural stimuli by producing far greater levels of dopamine release resulting in prolonged stimulation (Kelley and Berridge 2002).

#### Modeling Substance Abuse in Animals

Psychostimulants possess the ability to stimulate DA transmission in the nucleus accumbens (Di Chiara 2002). Studies using amphetamine have shown that the dopamine system is critically important for development and expression of behavioral sensitization (Kalivas et al. 1993a, 1993b). Acute or repeated amphetamine exposure results in augmented dopamine overflow in the striatum and nucleus accumbens (NAcc) in vivo (Wilkinson et al. 1993) and in vitro (Castaneda et al. 1988). Local administration of amphetamine in the NAcc in animals previously sensitized to amphetamine also produces increased DA overflow in NAcc and striatum (Pierce and Kalivas 1996).

### Dopamine System

Dopamine is estimated to constitute up to 80% of the total catecholamine content in the brain, but the total number of dopaminergic cells is relatively small (Lindvall et al. 1984, Williams and Goldman-Rakic 1998). Three major divisions of dopaminergic pathways innervate the forebrain and basal ganglia (Bjorklund and Lindvall 1984, Lewis et al. 1998, Tzschentke 2001). These cell groups are designated as A8, A9, and A10 and generally corresponds to the DA cells of the substantia nigra (A9), ventral tegmental area (A10), and the retrorubral area (A8) (Porrino and Goldman-Rakic 1982, Williams and Goldman-Rakic 1998). Dopaminergic neurons are functionally involved in higher motor execution, goal-directed behaviors including motivation, reward learning, prediction, and working memory (Egan and Weinberger 1997, Lewis et al. 1998). Impairment of these neurons results in neurological and psychiatric disorders (Goldman-Rakic 1998).

Ascending dopaminergic fibers innervate a large number of subcortical and cortical structures including the neocortex. Structures of the basal ganglia, particularly the striatum, receive the most dense dopaminergic input (Boyson et al. 1986). Within the neocortex, sensory areas are sparsely innervated while cortical areas such as the primary motor cortex, prefrontal cortex, and anterior cingulate cortex are more densely innervated by dopaminergic neurons (Berger and Gaspar 1994, Durstewitz et al. 1999). This innervation reflects the role of dopamine in motivation, reward, motor function, and executive function as compared to involvement in sensory systems.

There are several major dopaminergic pathways. The **nigrostriatal system** originates in the substantia nigra pars compacta, the ventral tegmental area, and the

retrorubral nucleus and projects to the caudate, putamen, globus pallidus, and nucleus accumbens. The nigrostriatal pathway is important in motor control (Feldman et al. 1996). The **mesolimbic system** contains the circuitry involved in reward and originates in the ventral tegmental area, substantia nigra, and retrorubral nucleus and projects to the septum, amygdala, hippocampus, nucleus of the diagonal band, anterior olfactory nucleus, nucleus accumbens, and olfactory tubercles (Fuxe 1985). The **mesocortical pathway** originates in the ventral tegmental area, projects to the frontal cortex, and is essential to the normal cognitive function of the prefrontal cortex (Feldman et al. 1986).

The majority of neurons in the NAcc are GABAergic and they project predominately to the ventral tegmental area (VTA) and the ventral pallidum, regions thought to be involved in the rewarding effects of most drugs of abuse (Self 2004). The GABA system modulates both dopamine and glutamate transmission within the NAcc and its areas of projection. Glutamatergic neurons converge on the same dendritic spines of medium-size spiny GABA neurons as dopaminergic afferents without making direct connections between the glutamatergic and dopaminergic projections (Segovia and Mora 2001). Pulvirenti and colleagues (1991) suggest that glutamate from the NAcc modulates stimulation at the level of the NAcc by interacting with the integrated output of the region.

### Dopamine Receptors

All known dopamine receptors are G-protein coupled, slow acting metabotropic receptors that functionally modulate other receptor systems and/or ion channels (Missale et al. 1998). Activation of these receptors, through intracellular cascades, modulates properties of synapses and postsynaptic neurons. There are five dopamine

receptor subtypes belonging to two families: the D1-like receptors and the D2-like receptors. Functionally, these two types of receptors have opposing actions in the cellular cascade. The D1-like receptors activate adenylyl cyclase resulting in an increase of cyclic adenosine monophosphate (cAMP). The D2-like receptors inhibit adenylyl cyclase resulting in a decreased concentration of cAMP (Greengard et al. 1999). The D1-like receptor family contains the D1 and D5 dopamine receptors, while the D2-like receptor family contains the D2, D3, and D4 dopamine receptors.

The distribution of the D1 receptors has been mapped by in vitro autoradiography using several agonists and antagonists. The greatest densities of D1 receptors are found in the caudate, putamen, NAcc, and olfactory tubercle. Lower densities are found in the neocortex, thalamus, cerebellum, hippocampus, septum, and hypothalamus (Boyson et al. 1986, Dawson et al. 1986, Wamsley et al. 1992, Duffy et al. 2000).

Activation of D1 dopamine receptors is necessary for the induction of sensitization by amphetamine in the VTA (Vezina 1996). Sensitization produced by amphetamine in the ventral tegmental area is blocked by systemic injection of the D1 antagonist SCH23390 (Vezina 1996). Pre-amphetamine administration of selective dopamine D1 or D2 antagonists have been shown to block amphetamine sensitization, although the D1 receptor has been shown to play a more important role than the D2, although this has not been thoroughly investigated (Vezina and Stewart 1989; 1996). Dopamine D1 receptors have a higher affinity for dopamine than D2 receptors (Seeman and Van Tol 1994). Systemic pretreatment with the D1 receptor antagonist SCH23390 inhibited the locomotor activating effects of repeated psychostimulant administration and prevented the development of sensitization (Hummel and Unterald 2002). Results

have also shown location specificity in the role of DI receptors in sensitization. It appears that noncortical DI receptors are more important than cortical DI receptors in the development of sensitization, intracortical administration of DI antagonist failed to significantly affect psychostimulant induced locomotor activity or stereotypy, whereas DI antagonist treatment within the ventral tegmental area has been shown to block sensitization (Beyer and Steketee 2002; Vezina 1996).

Dopamine D2 receptors are most densely located in the caudate, putamen, nucleus accumbens, olfactory tubercle, substantia nigra pars compacta, and the glomular layer of the olfactory bulbs with less dense populations in the central nucleus of the amygdala, lateral septum, superior colliculus, molecular layer of the hippocampus, and the entorhinal cortex (Boyson et al. 1986, Pinto and Sesack 2008).

Amphetamine induced sensitization may involve the D2 receptor both presynaptically at the autoreceptor, a receptor located on the presynaptic neuron terminal that serves as a part of a feedback loop in signal transduction, and at postsynaptic D2 receptors. Daily amphetamine treatment has been shown to produce a subsensitization of dopamine D2 autoreceptors in the ventral tegmental area (Seutin et al. 1991, Wolf et al. 1993, Muscat et al. 1996). The subsensitization of D2 autoreceptors occurs in conjunction with an increased activity of A10 dopaminergic neurons in the ventral tegmental area (Pierce et al. 1995). The increased activity in A10 neurons may result in priming of postsynaptic dopamine neurons (Ackerman and White 1992, Henry et al. 2000, Hopf et al. 2007). In response to amphetamine treatment, the postsynaptic signal transduction is linearly correlated to extracellular dopamine concentration. It has been suggested that at lower dopamine concentrations presynaptic binding may be

dominate to postsynaptic binding (Kuczenski and Segal 1989, Ren et al. 2009). Systemic administration of selective D2 antagonist (Vezina, 1989) has been shown to not affect amphetamine sensitization, and a less selective D2-like antagonist, eticlopride, that was also used in this study, has been infused into the ventral tegmental area and also shown to block amphetamine sensitization. However, there are no data on the systemic effects of eticlopride on amphetamine sensitization or its effects on dopamine overflow.

An important issue of the effects of neonatal quinpirole is the pre- and post-synaptic effects of this drug treatment. Quinpirole is a dopamine D2/D3 agonist and D2 as well as D3 receptor are located both pre- and post-synaptically. Blockade of postsynaptic receptors is generally considered to be the primary effect of neuroleptic administration and will result in a reduction of dopaminergic transmission. For some dopaminergic neurons, this immediate effect is opposed by compensatory feedback mechanisms (Galloway 1990). The blockade of presynaptic autoreceptors by neuroleptics results in an increased synthesis and release of dopamine from the dopaminergic terminal, thereby overcoming the blockade of dopamine receptors produced by the antagonist. There are also long-loop neuronal feedback pathways that can be involved. For example, during periods of enhanced postsynaptic dopamine receptor stimulation, these loops exert negative feedback effects on dopamine cell firing, whereas the blockade of postsynaptic receptors would produce a compensatory increase in dopamine cell firing (Benkert et al. 1992). Within the mesolimbic system, data have shown that either the D1 agonist SKF 38393 or the D2 agonist quinpirole can increase extracellular dopamine levels in the nucleus accumbens in a dose-dependent

fashion without affecting dopamine levels in the ventral tegmental area (Rahman and McBride 2001). Further, coadministration of SKF 38393 and quinpirole produce a concomitant reduction in the extracellular levels of dopamine in the nucleus accumbens as well as in the ventral tegmental area. Blockade of either the D1 or D2 receptor was sufficient to reverse the effect of the coinfusion of agonists. These results suggest that the activation of dopamine D1 or D2-like receptors are independently able to regulate local terminal dopamine release in the nucleus accumbens, but stimulation of both subtypes is required for activation of the negative feedback pathway to the ventral tegmental area (Rahman and McBride 2001).

### Sex Differences

Studies of sex differences in schizophrenia have concluded that women tend to have a less intrusive course of illness including less hospitalizations and better social functioning than men (Mueser and McGurk 2004). Studies have suggested that the later onset of schizophrenia in women is resultant of the effects of estrogen on D2 receptors in the central nervous system (CNS). Studies have shown that estrogen treatment reduced the affinity of sulpiride to the D2 receptor, leading to the conclusion that estrogen treatment reduced the sensitivity of D2 receptors (Hafnér et al. 1991a). Studies have also revealed an elevated vulnerability threshold to schizophrenia in women that persists until menopause, implicating the modulatory role of estrogen on the dopamine system (Hafner et al. 1998). In female schizophrenic patients, a correlation was found that the higher the estradiol level, the more 'normal functioning'<sup>1</sup> the behavior was judged to be (Reicher-Rössler et al. 1994). Additionally, it has been suggested that estrogen might act on NMDA receptors as well, both in the menstrual cycles and over a

longer period of time. This effect could contribute to the protective effect of estrogen in schizophrenia given the implications of the involvement of glutamate within the disease (Woolley and McEwen 1994).

#### Sex Hormones and Psychostimulant Abuse

Previous studies have indicated gender differences in the neurobiological response to psychostimulants and in behavioral sensitization (Becker 1999, Bowman et al. 1999). Sex differences are present for all phases of drug abuse: initiation, escalation of use, progression to addiction, withdrawal, and relapse (Haaren and Meyer 1991, Van Etten et al. 1999, Lynch and Carroll 2002, Carroll et al. 2004). While the rates of drug abuse are currently lower in women than men, once addicted to a drug, women show more difficulty in cessation of use than men (Lynch and Carroll 2002, Breese et al. 2005). In women, the subjective effects of psychostimulants, particularly d-amphetamine, vary across the menstrual cycle. Subjective positive effects including euphoria, increased energy, and intellectual efficiency were reported when estradiol levels are higher than progesterone levels during the follicular phase (Becker and Hu 2008). Administration of estradiol during the follicular phase further increases the subjective effects of d-amphetamine (Justice and De Wit 2000).

The acute behavioral response to psychomotor stimulants in rodents reflects both the sex difference (including locomotor response, self-administration, and drug sensitivity) and the modulatory role of gonadal hormones in males and females (Becker and Beer 1986, Van Hatesveldt et al. 1989, Bassar et al. 2000). Behavioral sensitization can also be differentially affected by gonadal steroid hormones. For example, females exhibit more robust sensitization than do intact males (Robinson et al.

1981, Robinson et al. 1982, Camp and Robinson 1988, Forgie and Stewart 1994).

Following ovariectomy of female rats, the expression of sensitization to AMPH is attenuated or completely suppressed (Robinson et al. 1982, Sicar and Kim 1999).

Estradiol treatment in ovariectomized rats enhances locomotor sensitization induced by AMPH or cocaine (Peris et al. 1991; Forgie and Stewart 1994). Studies have shown that female rats exhibit increased locomotor response to amphetamine treatment versus male rats (Becker 1999, Melnick and Dow-Edwards 2001). Female rats also show greater and more prolonged stereotyped behavior following amphetamine treatment and more amphetamine induced rotational behavior than males (Beatty and Holzer 1978, Robinson et al. 1980).

There are no sex differences in the number or binding characteristics of striatal D2 DA receptors in adult rats (Hruska and Dilbergeld 1980, Levesque and Di Paolo 1988, Teicher et al. 2003). Striatal D2 receptor density increases at the onset of puberty but is decreased significantly by adulthood in males. Periadolescent females show less overproduction and pruning of striatal D1 and D2 receptors (Andersen and Teicher 2000). There are sex differences in basal and amphetamine (AMPH)-stimulated striatal DA release in the absence of gonadal hormones. Results from *in vivo* microdialysis in freely moving rats show that the basal extracellular concentration of DA is twice as high in the striatum of castrated males as in ovariectomized females (Xiao and Becker 1994). Enduring differences in D1 receptor density may play a role in the greater incidence of substance abuse in males given the implied role of the D1 system in the development of addiction (Naldonado et al. 1993).

In cultured striatal neurons from embryonic mice, estradiol administration induces changes in adenylate cyclase activity that are stimulated by D1 and D2 dopamine receptor agonists by modifying the G-protein coupling process (Maus et al. 1989a, Maus et al. 1989b). Administration of physiological concentrations of estradiol to striatal slices directly stimulated DA release in vitro (Becker 1990). Estradiol works directly on the striatum to induce changes in DA release and DA receptor activity. Further studies have indicated that the effect of estradiol on striatal DA release may be mediated indirectly by the effect of estradiol on GABAergic striatal neurons (Becker and Hu 2008).

#### Estrus Cycle and Psychostimulants

It has been hypothesized that dopamine function in females may be influenced significantly by the fluctuation of ovarian hormones throughout the estrus cycle (Becker 1990). The estrus cycle of the female rat has three stages: proestrus, diestrus, and estrus. This cycle typically recurs every 4 days (Marcondes et al. 2002). Ovarian hormone fluctuations induce variation in behavioral and neurochemical responses to psychostimulant drugs. Behavioral estrus occurs simultaneous to vaginal estrus and therefore an increased estrogen level is present during this time as well a behavioral estrus. During behavioral estrus in female rats, amphetamine-induced dopamine release in the striatum and amphetamine behaviors are greater than on other days of the estrus cycle (Becker et al. 1980, Becker et al. 1982, Becker et al. 1989, Becker et al. 2001). Studies have shown that a greater behavioral response is elicited from female rats with stimulation of the striatal dopamine system during behavioral estrus as compared to diestrus (Becker et al. 1982, Kalivas and Stewart 1991).

### Goals of This Study

Research on psychosis has indicated a high comorbidity of substance abuse and psychosis. In those cases with comorbid substance abuse and schizophrenia the drugs of choice are often psychostimulants and more specifically amphetamine or nicotine. Therefore, further investigations into amphetamine sensitization in a putative model of psychosis may offer insight of the mechanisms involved in comorbidity.

## CHAPTER 2

### METHODS

This study uses neonatal quinpirole treatments from postnatal days 1-11 to produce life-long changes consistent with some aspects of schizophrenia. Past studies by Kostrzewa and colleagues (Nowak et al. 2001) have shown that neonatal quinpirole treatment results in long-term increases in sensitivity of D2-like receptors. Adult animals were then treated with either SCH23390 (a D1 receptor antagonist) or eticlopride (a D2-like receptor antagonist) in an amphetamine behavioral sensitization design, with animals given amphetamine or saline every other day over a 14-day period for a total of 7 drug treatments. Approximately 15 mins before each amphetamine or saline treatment, a D1 antagonist (SCH23390), D2 antagonist (eticlopride), or saline will be given to produce blockade of D1 and D2 receptors.

One day after sensitization is complete, a guide cannula will be surgically implanted. Six to 8 days later, an amphetamine or saline challenge will be given. However, a microdialysis probe will be placed into the nucleus accumbens core during this challenge. On the challenge, animals will be given the same antagonist and adulthood drug treatment that they received during behavioral testing on the challenge.

The goals of this study are to determine the role of the dopamine D1 and D2 receptors in the amphetamine-induced sensitization of rats neonatally treated with quinpirole. Additionally, the study investigated potential sex effects of treatments on females versus males. It is hypothesized based on past findings (Vezina 1989) that a dopamine D1 antagonist would block amphetamine behavioral sensitization and no

increase in locomotor activity would be seen following amphetamine sensitization. In terms of the effects on dopamine overflow, it is hypothesized that DI antagonism will completely block the effects of amphetamine on dopamine overflow based on past findings (Vezina 1996).

Additionally, a D2/D3 antagonist was hypothesized to reduce sensitization versus saline treated control animals, although not to the level of animals given a DI antagonist. The D2 receptor has been shown to not play a role in amphetamine sensitization (Vezina 1996, others), but antagonism of the D3 receptor has been shown to block amphetamine sensitization (Richtand et al. 2003). Based on this finding, it is hypothesized that antagonist treatment with the D2/D3 antagonist eticlopride will reduce sensitization although not to the levels of DI antagonism. It is hypothesized that pre-treatment with eticlopride will actually potentiate the effects of amphetamine on dopamine overflow based on the fact that eticlopride will block the D2 and D3 autoreceptors presynaptically, that should enhance dopamine release at the synapse. Information in Figure 1 describes the treatment groups for this study, beginning with neonatal treatment and including antagonist treatment as well as adult drug treatment. Neonatal treatments were given once daily PI -11. The antagonist treatments were administered approximately 15 minutes prior to the adult drug treatment. Following adult drug treatment (10 minutes after administration), animals were placed in locomotor arenas for testing.

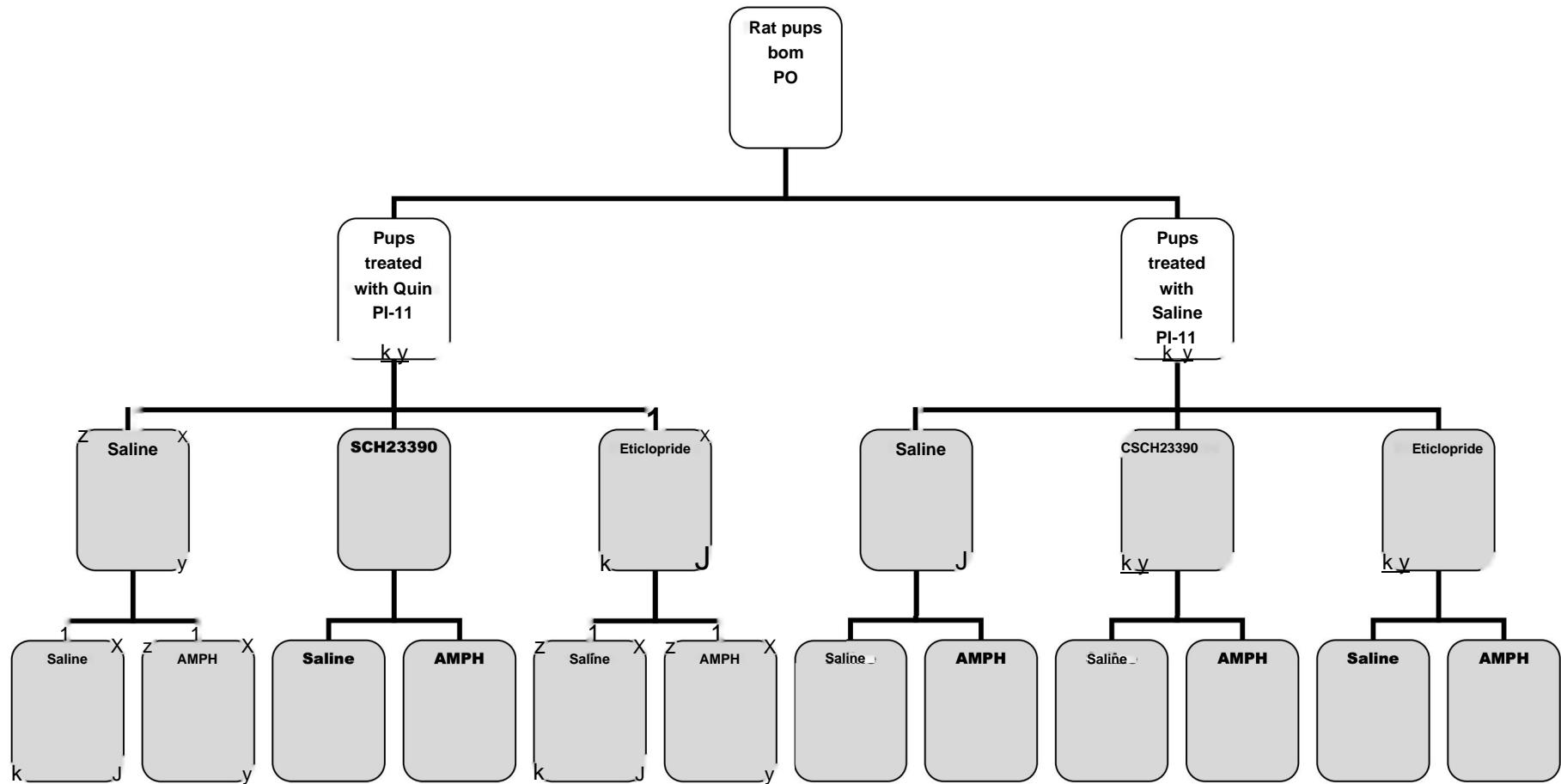


Figure 1 Diagram of drug treatments throughout the study. White boxes denote treatments during neonatal periods, prior to weaning. Gray boxes denote treatments involved in behavioral sensitization and microdialysis. AMPH denotes treatment with d-amphetamine.

### Subjects and Housing of Animals

A total of 10 untimed pregnant female Sprague Dawley rats were ordered from Harlan, Inc. (Indianapolis, IN). Once animals arrived, they were housed separately for 7 days. Throughout the experiment animals were kept in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited colony with a 12h light/dark cycle. All behavioral testing and neurochemical sampling occurred during the light cycle. After an initial quarantine period, the pregnant rats were housed singly until the pups were born, that was counted as postnatal day (P)0. Following birth, pups were housed with the female dams until P21. On P22, rats were weaned and housed in groups according to their neonatal treatments throughout adolescence and during amphetamine sensitization behavioral testing. Upon surgical implantation of the guide cannula, rats were singly housed for the remaining duration of the experiment.

### Neonatal Drug Treatment

Animals were injected with either quinpirole (1 mg/kg) or saline at a total volume of 1% of their body weight during treatment, PI -11. Following injections, the needle was left in place for approximately 5 seconds to avoid loss of drug as a result of capillary action resulting from needle removal.

### Drugs

All drug were administered through intraperitoneal (ip) injection, with the exception of postoperative ketoprofen that was given by intramuscular injection. For neonatal drug treatment and the yawning test quinpirole HCl (Sigma-Aldrich, St. Louis, MO) was administered. A dose of 1.0 mg/kg of the D2/D3 agonist quinpirole (or saline) was injected intraperitoneally (ip) for neonatal treatment. Previous studies have shown

this dose to be sufficient to produce an increase in dopamine D2 receptor sensitivity that will persist throughout the animal's lifetime (Kostrzewa et al. 1991, 1993, 1995). During behavioral sensitization a dose of 1.0 mg/kg d-amphetamine injected ip (Sigma-Aldrich, St. Louis, MO) was administered every other day over a 14-day period to adult rats. Previous data indicated that a dose of 1.0 mg/kg amphetamine was sufficient to produce increased dopaminergic overflow in animals neonatally treated with quinpirole. Additionally, this dose of amphetamine has been shown to produce behavioral sensitization (Hooks et al. 1992, Cadoni et al. 2002, Vanderschuren et al. 2003). For receptor antagonism, the D1 antagonist SCH 23390 (7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) was administered at a dose of 0.1 mg/kg (ip injection), that has been shown to be effective at blocking sensitization to a dose of 1.0 mg/kg amphetamine (Vezina 1996). The D1 antagonist, SCH 23390 has a >1000 times higher affinity at the D1 receptor as compared to the D2 receptor (Seeman and Grigoriadis, 1987). For D2 antagonism, a dose of 1.0 mg/kg eticlopride was administered. This eticlopride dose has been shown to block behavioral changes produced by 1.0 mg/kg doses of amphetamine (Vezina 1996). Eticlopride has  $>2.5 \times 10^6$  more affinity at the D2 receptor than the D1 receptor (Vezina 1996). The vehicle for all drug preparations was a 0.9% saline solution.

#### Yawning Behavior

At approximately P60, a time point equating to young adulthood in the rat, animals were tested for yawning behavior. Yawning has been shown to be a dopamine D2-like receptor mediated event. Studies have shown that this behavior may be mediated either by D2 (Cooper et al. 1989) or the D3 receptor subtype (Collins et al.

2005), although both of these receptors are members of the D2 receptor family. Prior to testing, all animals were administered quinpirole (100 pg/kg) and placed in an inverted cage without bedding. Bedding can result in chewing behavior that interferes with the observance and expression of the yawning behavior. Previous work indicated this dose of quinpirole resulted in the most robust yawning response within a 1 -hr period for each subject (Nowak et al. 2001). An observer blind to treatment condition observed the behavior for the period of 1 hour.

#### Habituation to the Locomotor Arena

Beginning at approximately 61 days of age, animals began habituation to the locomotor arena. For habituation, animals were placed in the locomotor arena for 10 minutes every 2nd day for 1 week. Immediately before each habituation session rats were administered an ip injection of saline. Animals were injected with saline to control for any locomotor effect that may exist as a result of the stress produced by the injection.

#### Behavioral Sensitization

Behavioral sensitization began approximately 2 days after the last habituation session. For each sensitization trial, approximately 10 minutes before injection of saline or amphetamine, rats were injected with the DI receptor antagonist SCH23390, the D2 receptor antagonist eticlopride, or saline. Ten minutes before placement in the arena, rats were administered with amphetamine (1.0 mg/kg) or saline based on group assignment. Once in the arena, horizontal activity was monitored for 10 minutes using an automated behavioral scanning system (AnyMaze, Stoelting Co, Dale Wood, IL). For horizontal activity measurements, the AnyMaze software digitally superimposes a 5

x 5 cm grid on the floor of the arena. Each time an animal crosses a line on the grid it is counted as a horizontal activity count. The total number of activity counts for each 10-min trial provided a quantification of the total horizontal activity.

As an additional behavioral measure turning behavior, both clockwise and counterclockwise was analyzed. Previous work has shown that rats demonstrate a drug-induced turning preference that is consistent in direction and correlated with the magnitude of turning when rats are retested with the same dose of the same drug (Becker et al. 1982, Glick et al. 1983). Turning behavior has been shown to be an effective measure of striatal function lending some additional information to the current study design (Robinson 1984).

#### Surgical Procedure for Implantation of Guide Cannula

On the day following the completion of amphetamine behavioral sensitization, a guide cannula (Bioanalytical Systems, Lafayette, IN) was surgically implanted, aimed at the nucleus accumbens core. Animals were first injected ip with a mixture of ketamine and xylazine for surgery to allow placement of the animal into the stereotaxic frame. However, the ratio of the ketamine/xylazine mixture was different based on the sex of the animal. Males were injected with a mixture of 60 mg/kg ketamine:8 mg/kg xylazine and females were injected with a mixture of 50 mg/kg ketamine: 10 mg/kg xylazine. These different doses helped to account for potential differences in metabolism of the anesthetic agents between male and female rats and to maintain proper levels of anesthesia during surgery (Zambricki and Dalecy, 2004). After the animal was anesthetized, the head was shaved and placed into the ear bars of a Kopf (Kopf Instruments, Inc., Tunjunga, CA) stereotaxic frame, and the nose of the animal was

inserted into a Kopf stereotaxic mask (Model 906, Kopf Instruments, Inc., Tunjunga, CA). The mask was connected to an anesthesia machine (JD Medical VT-100, Phoenix, AZ) that delivered an isoflurane/O<sub>2</sub> mixture (1-1.5%) at a rate of 2 L/min. An incision was made on the midline of the scalp and the covering connective tissue removed from the skull. A small hole was drilled through the skull at the following coordinates relative to Bregma: Anterior/Posterior = +1.0 mm, Medial/Lateral = +/-1.2 mm. The guide cannula was inserted through this opening to a dorsal level of 6 mm below bregma. These coordinates, taken from the stereotaxic atlas of Paxinos and Watson (1986) correspond to the dorsal location of the nucleus accumbens core. Three other small burr holes were drilled for the anchor screws and screws were attached to the skull. Dental acrylic was mixed (1.0 g powder, 2.0 ml\_ methyl methacrylate) and poured onto the skull and around the anchor screws to secure the cannula in place. Once the acrylic was dry, the incision was sutured closed and wound clips were placed over the sutures. After the wound was closed, the animal was removed from the stereotaxic frame and placed in an empty polycarbonate cage on a heating pad. Additionally, to ease postoperative discomfort, all animals were injected intramuscular with ketoprofen (5 mg/kg) and 10 cc of saline to replenish fluids lost during the surgical procedure. The animals were allowed to recover and returned to their home cage once ambulatory. Following microdialysis, the brains were analyzed by dissection for proper probe placement. Animals with incorrect probe placement were not included in the results.

### Estrus Cycle

On each day of testing as well as on the day of microdialysis, the state of estrus cycle of female rats was verified. A sample of cells was taken from the animal's vaginal lumen using a saline-moistened cotton swab. The sample was placed on a slide and analyzed under a microscope (Olympus microscope BH series). The stage of the estrus cycle was used as a variable in initial analysis of locomotor activity and dopamine overflow to determine if the stage of the estrus cycle impacted locomotor activity and/or dopamine overflow in the nucleus accumbens.

### Microdialysis and Amphetamine Challenge

Seven-10 days after amphetamine sensitization was complete, a microdialysis pin probe (Bioanalytical, Lafayette, IN) was implanted through the guide cannula with 2.0 mm of active membrane exposed. Prior to insertion of the probe, it was washed with artificial CSF for a period of 1 hour. Once the animal was attached to the probe, artificial CSF [145 mM sodium chloride, 2.7 mM potassium chloride, 1.0 mM magnesium chloride, 1.2 mM calcium chloride, 2.0 mM sodium phosphate at a pH of 7.4, Fisher Scientific] was perfused continuously at 2.0 pl/min through the dialysis probe for the duration of the procedure. Baseline samples were collected at 20-min intervals for 2 hours prior to drug administration. Following the baseline samples, rats were injected ip with saline, SCH 23390, or eticlopride, matching the antagonist treatment during sensitization. After the baseline period, d-amphetamine sulfate was injected using a cumulative dosing procedure similar to that used by Schad et al. (1996). Over the next four 20 minute intervals, rats were given an injection of the following doses of d-amphetamine in this order: 0.1 mg/kg, 0.4 mg/kg, 1.0 mg/kg, and 1.5 mg/kg. This

application results in a cumulative dose of 3.0 mg/kg of amphetamine by the end of the drug administration. Samples were collected every 20 min throughout drug treatment and 0.1 M perchloric acid was added to each vial to prevent catecholamine degradation. Samples were stored at -80 degrees C for later analysis.

#### HPLC Analysis

In preparation for analysis, several concentrations of dopamine (DA), 3,4 dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and norepinephrine (NE) were prepared in order to construct a standard curve from that the concentrations of catecholamines could be calculated. Before analysis, samples were thawed. Samples were injected into vials for analysis using an autosampler (ESA, Chelmsford, MA). The autosampler injected 40 pl of each sample onto the detection column allowing the sample to separate into its constituents that cleared the column at specific retention times. The area of each target peak was compared to the standard curve to calculate the concentration for each compound. The baseline concentrations of DA, DOPAC, HVA, and NE were all calculated in picograms (pg) per 10 pl. For microdialysis of all compounds following drug treatment, the percentage of baseline was calculated based on the concentration of the target compound at each time point.

#### Statistical Analysis

Analysis of variance (ANOVA) was the primary statistical test with a Fischer's post-hoc test used for statistical analysis of all significant interactions. The statistical significance was set at p<0.05 for all analyses. In the research design, there were four independent variables: neonatal drug treatment (between subjects, 2 levels: quinpirole or saline); antagonist treatment (between subjects, 3 levels: SCH23390, eticlopride, or

saline) adulthood drug treatment (between subjects, 2 levels: amphetamine or saline) and sex (between subjects, 2 levels: male or female). Regarding microdialysis, the time points were used as a repeated measure for the ANOVA analysis with regard to drug treatment (neonatal, antagonist, and adult drug treatment) and expressed as a percentage of baseline levels. Baseline levels were calculated by averaging the dopamine overflow concentrations of the three samples from the last hour of baseline collection. The drug treatment samples were then calculated as a percentage of the baseline with each animal calculated independently. For this analysis, a  $2 \times 2 \times 3 \times 9$  four-way ANOVA was initially used, with sex dropped as a variable when no sex differences were found. Table 1 outlines the subject numbers and treatments for the duration of the study.

Table 1 Numbers and sexes of subjects and the treatment groups for treatment throughout the study design. Neonatal treatments were given PI -11. The antagonist treatments were administered during adulthood on behavioral testing days and on the day of microdialysis.

Subjects	Neonatal treatment	Antagonist treatment	Adult drug treatment
8 Males, 8 Females	Saline	Saline	Saline
8 Males, 8 Females	Saline	Saline	Amphetamine
8 Males, 8 Females	Quinpirole	Saline	Saline
8 Males, 8 Females	Quinpirole	Saline	Amphetamine
8 Males, 8 Females	Saline	SCH23390	Saline
8 Males, 8 Females	Saline	SCH23390	Amphetamine
8 Males, 8 Females	Quinpirole	SCH23390	Saline
8 Males, 8 Females	Quinpirole	SCH23390	Amphetamine
8 Males, 8 Females	Saline	Eticlopride	Saline
8 Males, 8 Females	Saline	Eticlopride	Amphetamine
8 Males, 8 Females	Quinpirole	Eticlopride	Saline
8 Males, 8 Females	Quinpirole	Eticlopride	Amphetamine

## CHAPTER 3

## RESULTS

### Amphetamine Sensitization

#### Horizontal Activity

A repeated measures five-way ANOVA was used as the primary statistical measure. There were a total of five factors in the experimental design: sex (2 levels: male, female), neonatal drug treatment (2 levels: quinpirole, saline), antagonist treatment (3 levels: saline, SCH23390, eticlopride), adult drug treatment (2 levels: amphetamine, saline), and day of testing (repeated measure: day 1, day 7). Subjects were behaviorally tested for 7 days, but analysis included only days 1 and 7 to compare the beginning and end of drug treatment. The analysis of the microdialysis experiments revealed no sex differences and data were consolidated over the sex variable. Across all behavioral analyses there were no significant effects of estrus cycle stage; therefore, data analyses were collapsed across that variable. A 2 (sex) X 2 (neonatal drug treatment) x 2 (adulthood drug testing) x 2 (day of testing) x 3 (antagonist treatment) five-way ANOVA revealed significant main effects for antagonist treatment,  $F(1,143) = 121.21$ , pcO.OOI, adult drug treatment,  $F(1,143) = 35.19$ , pcO.OOI, and day of treatment,  $F(1, 143) = 4.90$ ,  $p < 0.029$ . There were six significant two-way interactions: neonatal drug treatment x day of testing,  $F(1,143) = 4.867$ ,  $p < 0.029$ , antagonist treatment x day of testing,  $F(2, 143) = 10.65$ , pcO.OOI, adult drug treatment x day of testing,  $F(1,143) = 4.39$ , pcO.OOI, neonatal drug treatment x antagonist treatment,  $F(2, 143) = 6.70$ ,  $p < 0.002$ , neonatal drug treatment x adult drug treatment  $F(1, 143) = 12.63$ , pcO.OOI, and antagonist treatment x adult drug treatment,  $F(2, 143) = 15.96$ , pcO.OOI. There

were also two significant three-way interactions of neonatal drug treatment x antagonist treatment x adult drug treatment,  $F(2, 143) = 3.10$ ,  $p<0.048$  and sex x neonatal treatment x antagonist  $F(2,143) = 2.844$ ,  $p<0.043$ . Table 2 indicates treatment codes for analysis.

Table 2 Treatment codes and abbreviations for groups.

Neonatal treatment	Antagonist Treatment	Adult Drug Treatment	Group Abbreviation
Saline	Saline	Saline	SSS
Saline	Saline	Amphetamine	SSA
Quinpirole	Saline	Saline	QSS
Quinpirole	Saline	Amphetamine	QSA
Saline	Eticlopride	Saline	SES
Saline	Eticlopride	Amphetamine	SEA
Quinpirole	Eticlopride	Saline	QES
Quinpirole	Eticlopride	Amphetamine	QEA
Saline	SCH23390	Saline	SIS
Saline	SCH23390	Amphetamine	S1A
Quinpirole	SCH23390	Saline	QIS
Quinpirole	SCH23390	Amphetamine	QI A

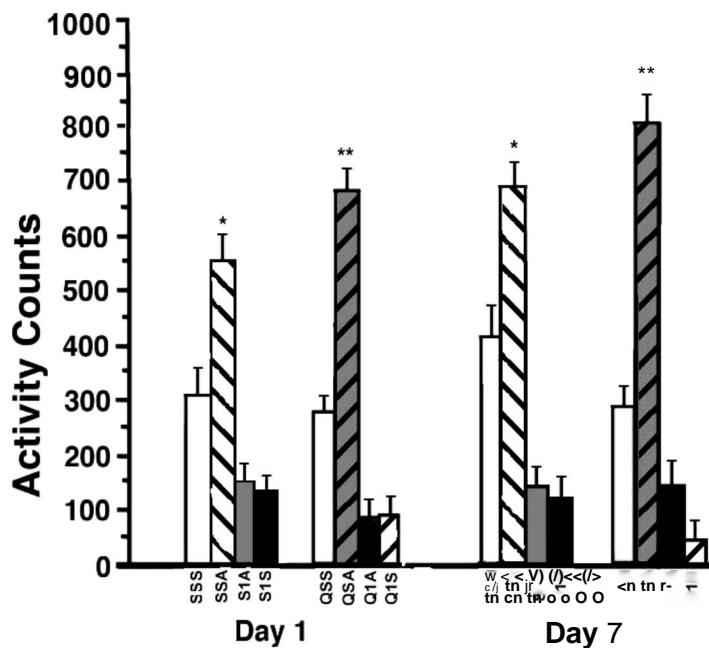
Analysis of the significant three-way interaction of neonatal drug treatment x antagonist treatment x adult drug treatment revealed that Group QSA demonstrate higher levels of activity than all other groups across both days of analysis. This finding demonstrates that neonatal quinpirole treatment produced a significant enhancement of the horizontal activity response to amphetamine, and this was blocked by either SCH 23390 or eticlopride. Analysis of the significant three-way interaction of sex x neonatal drug treatment x antagonist showed that SCH23390 and eticlopride were both effective

at blocking amphetamine sensitization in males, but in females only SCH 23390 was effective, as Group SSA and SEA were equivalent at days 1 and 7 and both of these groups were significantly higher than controls (Group SSS), figures 2 and 3. Therefore, it appears that males demonstrate an increased sensitivity to a D2-like antagonist as compared to females. Of course, this conclusion should be treated with some caution because only one dose of each antagonist was tested.

Analysis of the significant two-way interactions revealed that neonatal quinpirole treatment produced a significant increase in horizontal activity on day 7 as compared to day 1. SCH 23390 produced a significant decrease in activity compared to controls on days 1 and 7, whereas eticlopride actually produced a significant increase in horizontal activity compared to controls at days 1 and 7. Amphetamine produced a significant increase in horizontal activity at day 7 as compared to day 1. SCH 23390 was more effective at blocking the effects of neonatal quinpirole treatment than eticlopride, likely due to eticlopride's blockade of inhibitory D2-like autoreceptors. Neonatal quinpirole treatment enhanced the locomotor activity response to amphetamine compared to animals given neonatal saline. Finally, SCH23390 was more effective at blocking the effects of amphetamine compared to eticlopride, as expected, and this is supported by past work (Vezina, 1996). Analysis of the significant main effects revealed that SCH23390 reduced activity levels more effectively than eticlopride treatment and also prevented amphetamine-induced sensitization, supporting previous literature (Vezina 1996). Finally analysis of the significant main effect of adulthood drug treatment revealed that d-amphetamine significantly increased horizontal locomotor activity across days of testing as compared to saline controls, as expected. In sum, it appears that the

DI antagonist SCH 23390 was effective at blocking amphetamine sensitization and producing locomotor hypoactivity, whereas the D2-like receptor antagonist eticlopride blocked amphetamine sensitization, but produced less locomotor hypoactivity in females as compared to males, and did not produce hypoactivity compared to controls. This was to be expected, as eticlopride blocks the inhibitory dopamine D2 autoreceptor and therefore increases dopaminergic activity, that would be expected to produce an increase in locomotor activity.

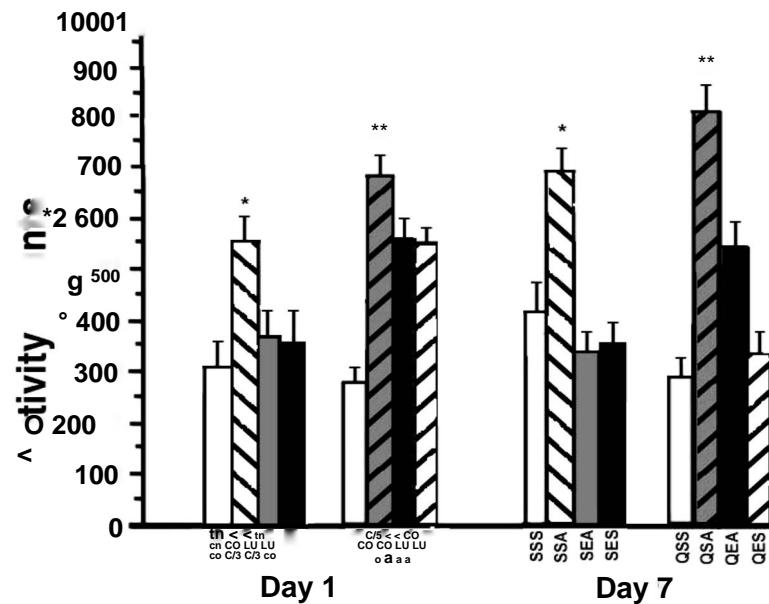
A) SCH23390 - males



\* indicates a significant difference in the saline-saline-amphetamine group  
 \*\* indicates a significant difference from all other groups in the response of the quinpirole-saline-amphetamine group

Figure 2 A) Horizontal activity of male subjects treated with SCH23390.

B) Eticlopride - males

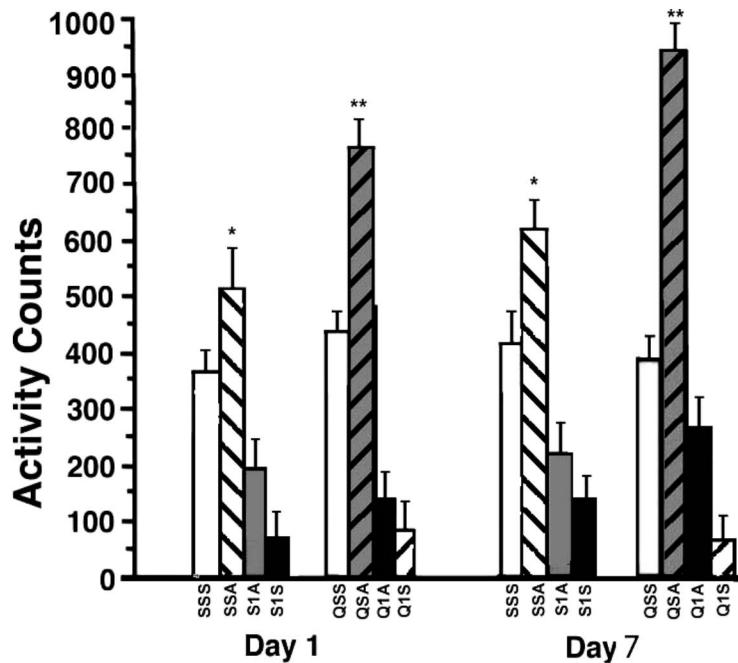


\* indicates a significant difference in the saline-saline-amphetamine group

\*\* indicates a significant difference from all other groups in the response of the quinpirole-saline-amphetamine group

Figure 2 B) Horizontal activity of male subjects treated with eticlopride. The QSA group had significantly different activity levels than all other groups on both days 1 and 7. SSA animals showed significantly higher activity levels than saline-treated control animals. SCH23390 was effective at lowering activity levels in all treatment constructs to below control levels. Data are presented as means of groups +/- SEM.

A) SCH23390 - females

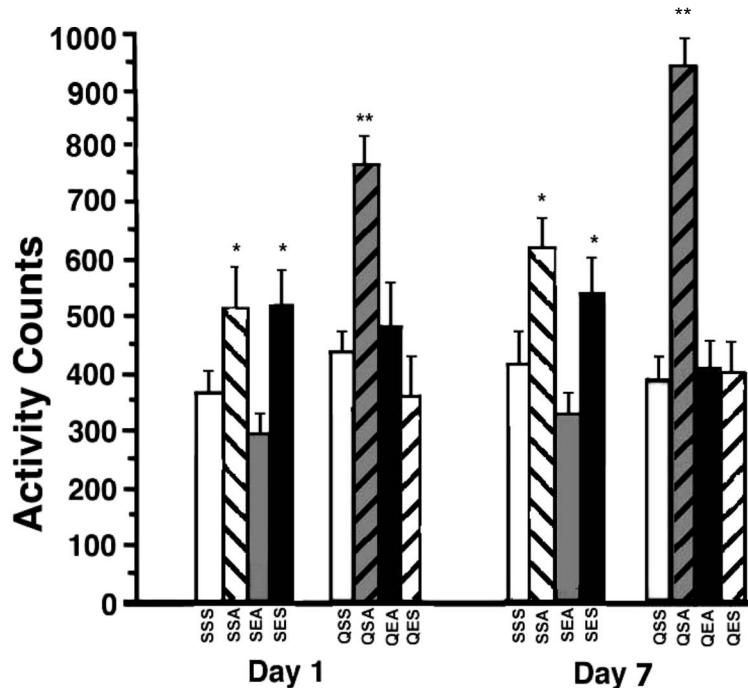


\* indicates a group with a significant difference from control

\*\* indicates a group with a significant difference from all other groups

Figure 3 A) Horizontal activity of female subjects treated with SCH23390.

B) Eticlopride - females



\* indicates a group with a significant difference from control

\*\* indicates a group with a significant difference from all other groups

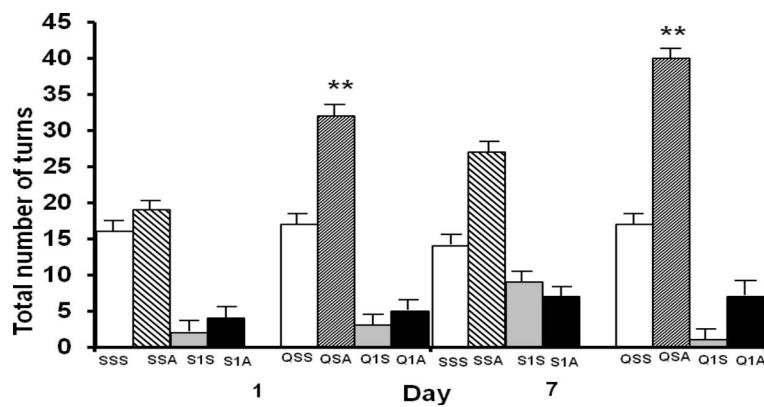
Figure 3 B) Horizontal activity of female subjects treated with eticlopride. SSA animals demonstrated a significant increase in activity over control animals. SCH23390 treated groups exhibited lower levels of horizontal activity compared to control animals. The SES group showed significantly greater activity than control animals on both days 1 and 7, with levels similar to the SSA group. Data are presented as means of each group +/- SEM.

### Turning Behavior

Results are shown in figures 4 (males) and 5 (females). For turning behavior, an initial analysis revealed no directional differences, so total (clockwise and counterclockwise) turns were summed and averaged for each group on days 1 and 7. Turning is presented as a function of neonatal drug treatment, antagonist drug treatment, adult drug treatment, and day of testing in Figure 3. A<sub>2</sub>x<sub>2</sub>x<sub>2</sub>x<sub>2</sub>x<sub>2</sub> five-way ANOVA revealed a significant main effect of antagonist drug treatment  $F(1,162) = 98.81$ ,  $p < 0.001$ , day of treatment  $F(1,126) = 9.637$ ,  $p < 0.002$  and adult drug treatment  $F(1,162) = 6.395$ ,  $p < 0.013$ , one significant three-way interaction of Day X Antagonist Treatment X Adult Drug Treatment  $F(1,162) = 3.802$ ,  $p < 0.025$ , one significant four-way interaction: Day X Sex X Neonatal Treatment x Antagonist Treatment  $F(1,162) = 3.915$ ,  $p < 0.05$ , and a significant five-way interaction of Day of Treatment X Sex X Neonatal Treatment X Antagonist Treatment X Adult Drug Treatment  $F(1,162) = 4.242$ ,  $p < 0.041$ . Analysis of the significant five-way interaction demonstrated that females given neonatal quinpirole, saline as antagonist treatment and amphetamine as adult drug treatment demonstrated a significantly higher amount of turning behavior as compared to all other groups on the 1st day of treatment (day 1). Both SCH23390 and eticlopride blocked increases in turning behavior produced by amphetamine, and SCH23390 was more effective at blocking increases in turning behavior produced by amphetamine as compared to eticlopride, consistent with the horizontal activity results. Analysis of the significant four-way interaction again revealed that females given neonatal quinpirole treatment and saline as antagonist treatment demonstrated significantly higher amount of turning behavior on day 1. Post hoc analysis of the significant three-way interaction

revealed that animals given saline as antagonist treatment and amphetamine as adult drug treatment demonstrated significantly higher levels of turning behavior on day 7 compared to all other groups. The significant main effects revealed that SCH23390 blocked the increase in turning behavior more so than eticlopride, although both significantly reduced turning behavior as compared to saline treated control subjects, and amphetamine significantly increased turning behavior. It appears that D2 priming as is produced by neonatal quinpirole treatment enhanced amphetamine-induced turning behavior, but this effect was blocked by either D1 or D2 antagonism.

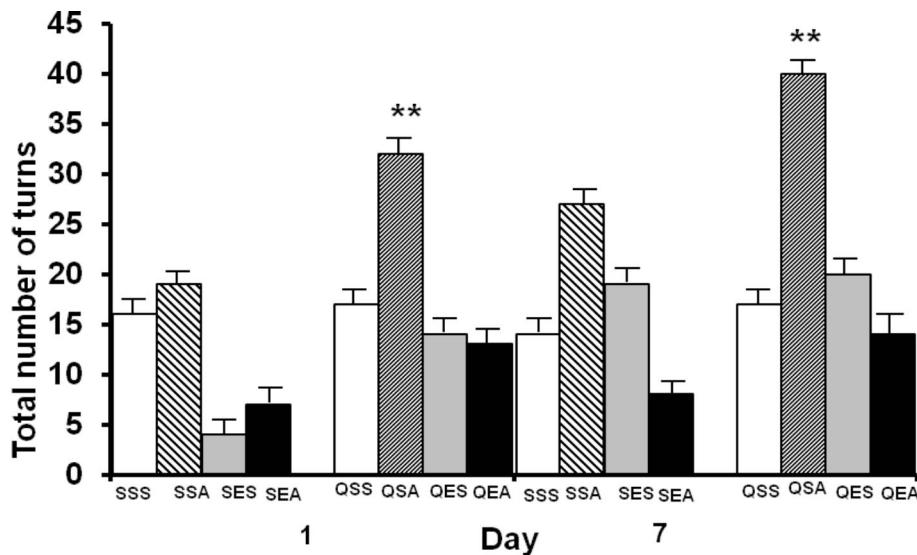
A) Males SCH23390



\* indicates a group with a significant difference from control  
 \*\* indicates a group with a significant difference from all other groups

Figure 4 A) Turning behavior of male subjects treated with SCH23390.

B) Males Eticlopride

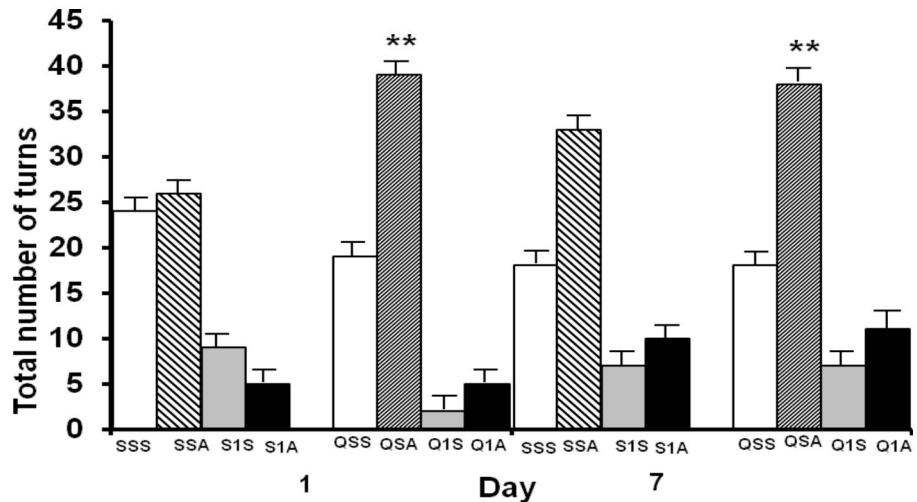


\* indicates a group with a significant difference from control

\*\* indicates a group with a significant difference from all other groups

Figure 4 B) Turning behavior of male subjects treated with eticlopride. SSA animals demonstrated a significant increase in turning over control animals. SCH23390 treated groups exhibited lower levels of horizontal activity compared to control animals. The QSA group showed significantly greater activity than all other groups on both days 1 and 7. Data are presented as the mean of each group +/- SEM.

A) Female SCH23390

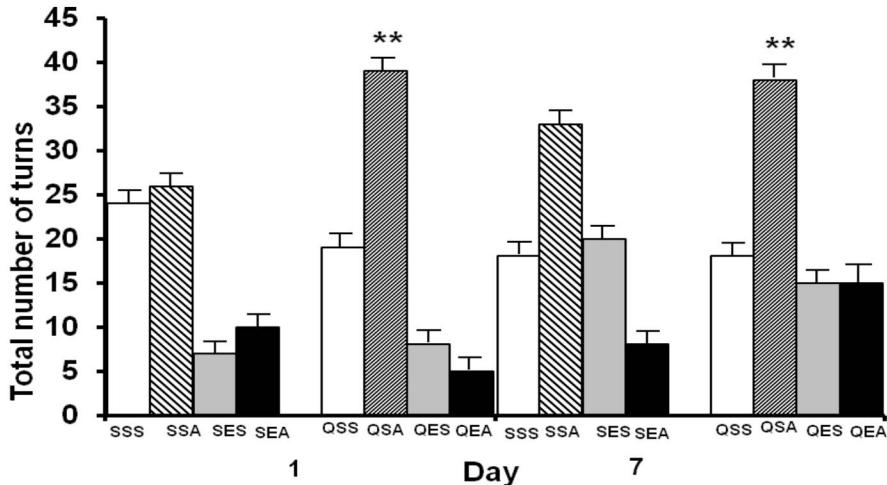


\* indicates a group with a significant difference from control

\*\* indicates a group with a significant difference from all other groups

Figure 5 A) Turning behavior of female subjects treated with SCH23390.

## B) Female Eticlopride



\* indicates a group with a significant difference from control

\*\* indicates a group with a significant difference from all other groups

Figure 5 B) Turning behavior of female subjects treated with eticlopride. SSA animals demonstrated a significant increase in turning over control animals on Day 7. SCH23390 treated groups exhibited lower levels of turning compared to control animals. The QSA group showed significantly greater activity than all other groups on both days 1 and 7. Data are presented as group means +/- SEM.

### Microdialysis

As with behavior, there were a total of five factors in the experimental design: sex (male, female), neonatal drug treatment (2 levels: quinpirole, saline), antagonist treatment (3 levels: saline, SCH23390, eticlopride), adult drug treatment (2 levels: amphetamine, saline), and time point after drug administration (repeated measure: time point after drug administration). Across all analyses regarding DA, DOPAC, HVA, and NE, there were no significant sex differences revealed and data were collapsed across that factor for analysis. Thus, a four-way repeated measures ANOVA was the primary

statistical measure for analysis of dopamine levels in the nucleus accumbens core. Additionally, initial analysis also revealed no significant effect of estrus cycle stage, so data were collapsed over that factor as well.

### Dopamine Overflow

Dopamine overflow in the nucleus accumbens core is presented as a function of adult drug treatment and antagonist drug treatment in figures 6 (SCH23390) and 7 (eticlopride).

A four-way repeated measures ANOVA revealed three significant main effects: time point  $F(8,84) = 2.46$ ,  $p < 0.012$ , antagonist drug treatment  $F(2,84) = 9.41$ ,  $p < 0.001$  and adult drug treatment  $F(1,84) = 6.24$ ,  $p < 0.014$ , as well as four significant two-way interactions: neonatal drug treatment x antagonist drug treatment  $F(2,84) = 4.61$ ,  $p < 0.013$ , neonatal drug treatment x adult drug treatment  $F(1,84) = 4.58$ ,  $p < 0.04$ , antagonist drug treatment x adult drug treatment  $F(2,84) = 12.77$ ,  $p < 0.001$ , time point x antagonist drug treatment  $F(8,84) = 2.77$ ,  $p < 0.001$  and one significant three-way interaction time point x antagonist drug treatment x adult drug treatment  $F(16,84) = 2.67$ ,  $p < 0.001$ . Post hoc analysis of the significant three-way interaction revealed that amphetamine produced a significant increase in dopamine overflow in animals given saline for antagonist drug treatment at the 20-180 min time points. Post hoc analyses of the significant two-way interactions revealed that neonatal quinpirole treatment coupled with saline treatment for the antagonist drug treatment significant increased dopamine overflow; neonatal quinpirole treatment enhanced dopamine overflow in animals treated with amphetamine; rats given saline during antagonist drug treatment coupled with

amphetamine for adult drug treatment demonstrated a significant increase in dopamine overflow, and saline during antagonist treatment produced a significant increase in dopamine overflow at time points 20-180 min after drug treatment. Amphetamine treatment resulted in a significant increase in dopamine overflow as expected. Neonatal quinpirole treatment followed by adult d-amphetamine administration resulted in the greatest increase in dopamine overflow (Nowak et al. 2001, Cope et al. 2008). Thus D2 priming appears to potentiate the increase in dopamine overflow seen following adult amphetamine administration. It appears that both SCH23390 and eticlopride can effectively attenuate the D2 priming effect that results in increased dopamine overflow in the nucleus accumbens. This may be the result of a lack of D1/D2 synergism in the presence of antagonism of either the D1 or D2 receptor.

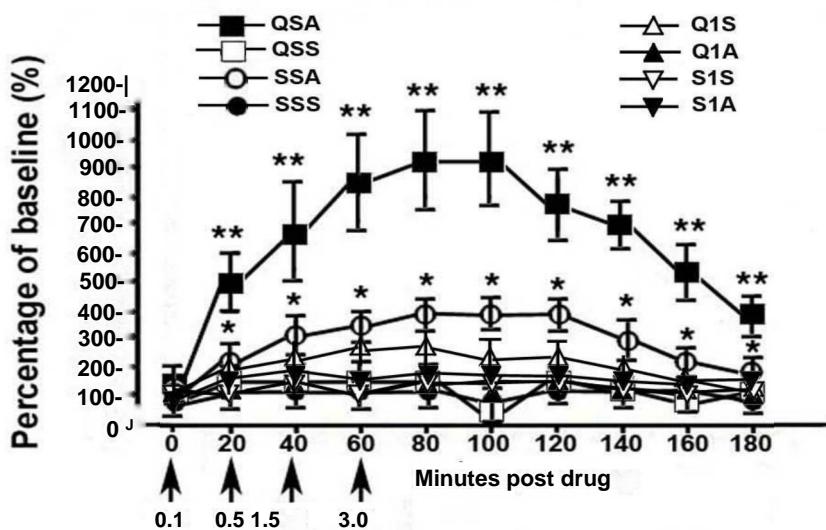


Figure 6 Dopamine overflow in subjects treated with SCH23390. Group QSA exhibited significantly higher levels of dopamine in samples than all other groups. Group SSA also exhibited increased dopamine levels compared to antagonist treated subjects. A single \* represents a significant difference from saline-treated control subjects (SSS). \*\* represent a significant difference from all other groups. Arrows indicate cumulative

dosing of amphetamine as the experiment progressed. Values are means for each group +/- SEM.

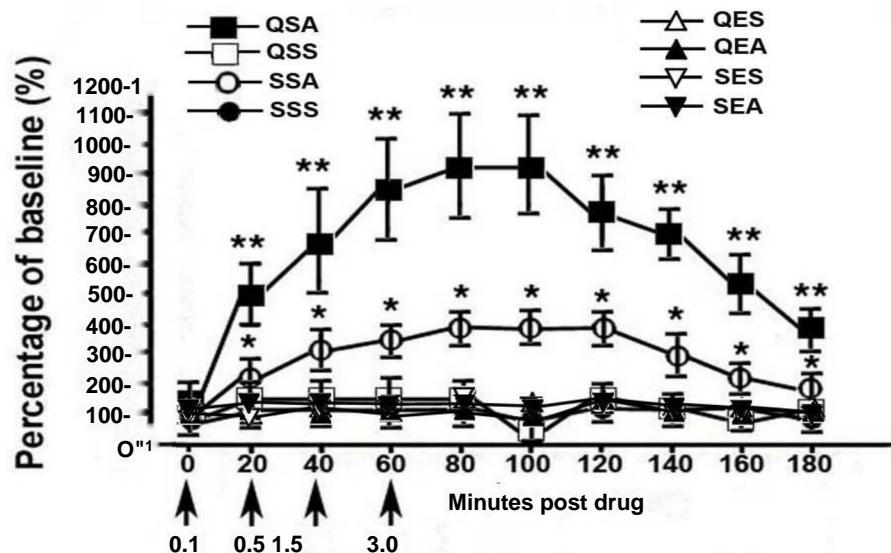


Figure 7 Dopamine overflow in subjects treated with eticlopride. Eticlopride treated subjects demonstrated no significant difference in dopamine levels compared to control subjects. A single \* represents a significant difference from control subjects. \*\* represent a significant difference from all other groups. Arrows indicate cumulative dosing of amphetamine as the experiment progressed. Values are means for each group +/- SEM.

#### DOPAC Overflow

A four-way repeated measures ANOVA revealed two significant main effects: time point  $F(8,96) = 2.41$ ,  $p<0.014$ , adult drug treatment  $F(1,96) = 5.75$ ,  $p<0.018$ , and one significant three-way interaction neonatal drug treatment x antagonist drug treatment x adult drug treatment  $F(2,96) = 3.79$ ,  $p<0.026$ . Post-hoc analysis of the significant three-way interaction revealed that neonatal quinpirole treatment coupled with SCH-23390 and amphetamine treatment in adulthood produced a significant increase in DOPAC overflow, data represented in table 3. Neonatal quinpirole

treatment resulted in an increase in DOPAC, and SCH 23390 was unable to reduce DOPAC overflow to control levels. Taken together, these results indicate that D2 priming facilitates DOPAC overflow. No other significant differences were found between groups. Analysis of the significant main effects revealed that there were significant changes in DOPAC over the different time points, and amphetamine significantly increased DOPAC compared to rats treated with saline.

Table 3 DOPAC Microdialysis. DOPAC results from microdialysis presented as an average concentration for each group following adult drug treatment (AMPH or saline). Values are DOPAC concentrations in picograms. A \* denotes a significant difference from saline-treated control animals (SSS).

<u>Group Abbreviation</u>	<u>Mean Concentration</u>
Q1A	128.09*
QIS	96.54
QEA	90.22
QES	145.56*
QSA	81.66
QSS	321.53
S1A	80.79
SIS	113.34
SEA	89.12
SES	135.92
SSA	80.02
SSS	87.18

### Norepinephrine Overflow

A four-way repeated measures ANOVA revealed a significant main effect of time point  $F(8,80) = 2.20$ ,  $p<0.026$ , three significant two-way interactions: time point x neonatal treatment  $F(1,80) = 5.37$ ,  $p<0.023$ , time point x antagonist treatment  $F(2,80) = 6.83$ ,  $p<0.002$ , time point x adult drug treatment  $F(1,80) = 8.35$ ,  $p<0.005$  and a significant four-way interaction of time point x neonatal treatment x antagonist treatment x adult drug treatment  $F(2,80) = 3.53$ ,  $p<0.034$ . Post hoc analysis of the significant four-way interaction revealed that rats given neonatal quinpirole treatment, saline as their antagonist drug treatment, and amphetamine in adulthood produced a significant increase in NE overflow at 80, 100, and 120 min post drug treatment, data shown in figures 10 and 11. Interestingly, saline-treated controls demonstrated significantly greater levels of norepinephrine at the 20 and 40 minute time points following drug administration, but this may also been due to unstable baseline in this group (data not shown). Overall, amphetamine treatment without antagonism produced a significant 200% increase in NE levels at 80, 100, and 120 minutes post drug treatment in the nucleus accumbens core compared to controls, consistent with previous work (McKittrick and Abercrombie 2007). Antagonist treatment with either SCH23390 or eticlopride blocked the effects of amphetamine on NE with levels equal to saline treated controls (Group SSS).

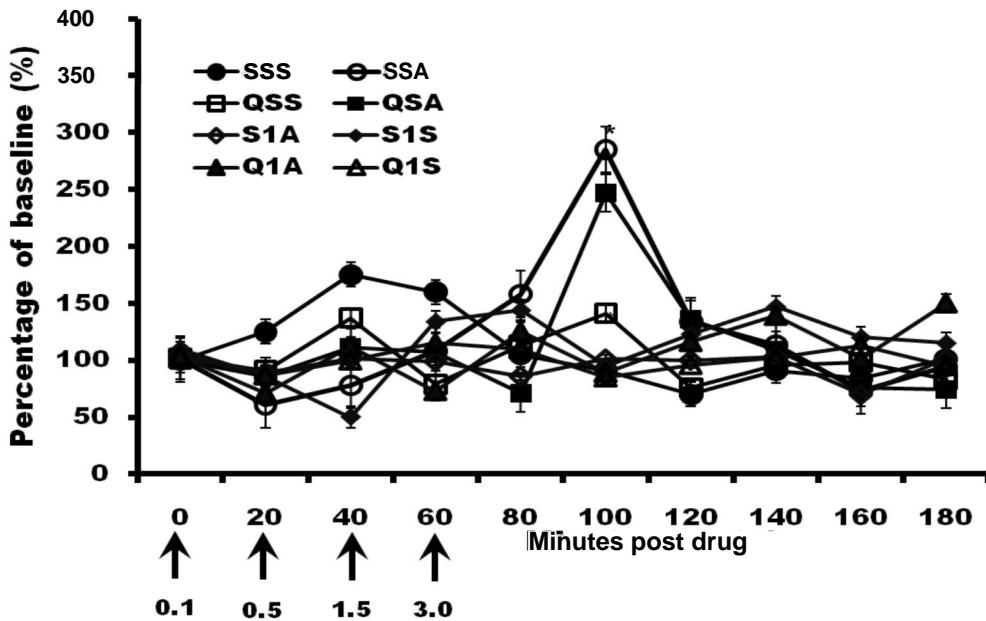


Figure 8 Norepinephrine overflow in subjects treated with SCH23390. All groups demonstrated significantly lower levels of norepinephrine for the first 60 minutes post drug as compared to saline-treated control subjects. At 100 minutes post drug treatment, groups SSA, QSA and QSS exhibited higher levels of norepinephrine than saline-treated control subjects. Arrows represent dosage of amphetamine in mg/kg administered at each time point. Data are represented by percentage of baseline +/- SEM. A \* represents a significant difference from saline-treated control subjects (SSS).

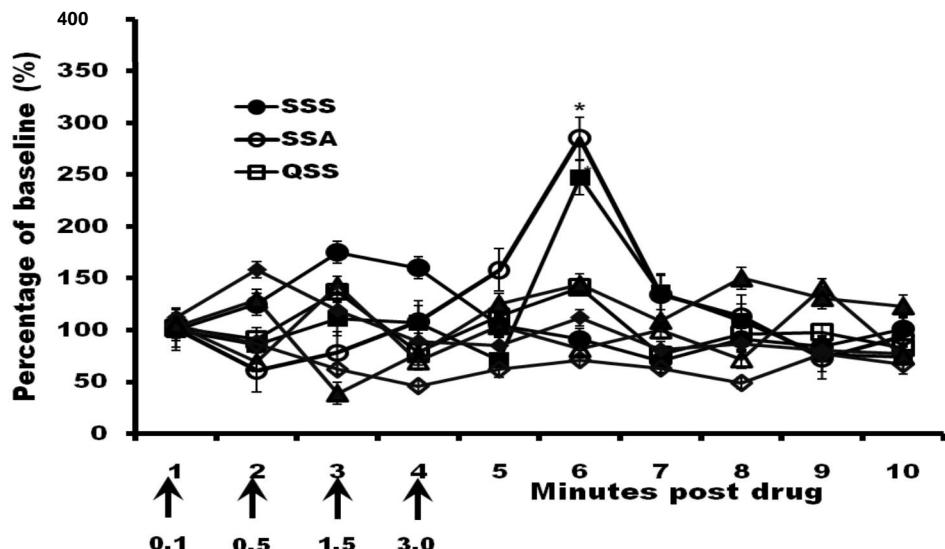


Figure 9 Norepinephrine overflow in subjects treated with eticlopride. Groups SSA and QSA exhibited significantly higher levels of norepinephrine compared to control subjects. Group QEA exhibited significantly higher levels of norepinephrine compared to control groups. No other significant differences were found. Arrows represent dosage of amphetamine in mg/kg administered at each time point. Data are represented by percentage of baseline +/- SEM. A \* represents a significant difference from saline-treated control subjects (SSS).

#### Homovanillic Acid Overflow

A<sub>2</sub>x2x3x9 revealed no main effects or interactions of drug treatment in the levels of homovanillic acid (HVA) during baseline or after drug treatment.

## CHAPTER 4

### DISCUSSION

The results of this study indicate several important findings. First, neonatal treatment with the D2/D3 receptor agonist quinpirole enhanced sensitization to amphetamine in adulthood. Second, neonatal quinpirole treatment also enhanced the dopaminergic response to amphetamine in adulthood in the nucleus accumbens core. Third, it appears that both the D1 and D2 receptors are differentially involved in amphetamine-induced sensitization. Based on findings that D2 primed animals exhibited an increased locomotor response and dopamine overflow in a brain area associated with drug reward, this study indicates that enhancing dopamine D2 receptor sensitivity enhances the behavioral and dopaminergic response to amphetamine, that presumably increases the rewarding effect of the drug in D2 primed animals. This is consistent with previous studies that have shown neonatal quinpirole treatment produces a more robust sensitization to the psychostimulant nicotine in adolescence and adulthood and produces a more robust dopaminergic response to amphetamine in adulthood, that is consistent with the effects reported here, because both amphetamine and nicotine are in the psychostimulant drug family (Perna et al. 2008, Cope et al. 2009).

In terms of the roles of D1 and D2 receptors, although both D1 and D2 antagonism blocked amphetamine sensitization in males and dopamine overflow, pretreatment with the D1 receptor antagonist SCH-23390 was more effective in producing a reduction in locomotor activity in males and dopamine overflow than the D2-like receptor antagonist eticlopride. Pre-amphetamine treatment with the D1

antagonist SCH 23390 blocked both locomotor sensitization to amphetamine as well as accumbal dopamine overflow, consistent with our hypothesis. Previous work has shown that DI receptors are necessary for the induction of behavioral sensitization (Vezina 1996). Results here confirm that the blockade of the DI receptor with SCH23390 is sufficient to prevent amphetamine-induced behavioral sensitization. It has been postulated that induction of enduring enhancements in the ventral tegmental area (VTA) induced by amphetamine involve the somatodendritic release of dopamine and its action at dopamine DI receptors. Dopamine DI receptors in the VTA appear to be positioned such that they can exert a critical influence on the initiation of changes in the reactivity of accumbal dopaminergic neurons that lead to a long-term sensitized locomotor response as well as dopaminergic response from the nucleus accumbens (Vezina 1996). The release of both GABA and glutamate into the ventral tegmental area has also been shown to be modulated by DI receptors (Cameron and Williams 1993, Kalivas and Duffy 1995), and both of these neurotransmitters have been shown to influence the induction of behavioral sensitization to psychostimulants (Wolf et al. 1994). Thus, the induction of sensitization may depend on DI mediated release of neurotransmitters in addition to dopamine. The drug reward pathway including the ventral tegmental area and nucleus accumbens are represented in figure 10.

The box denotes the brain region containing the reward circuitry. The lines within the box represent the mesolimbic pathway.

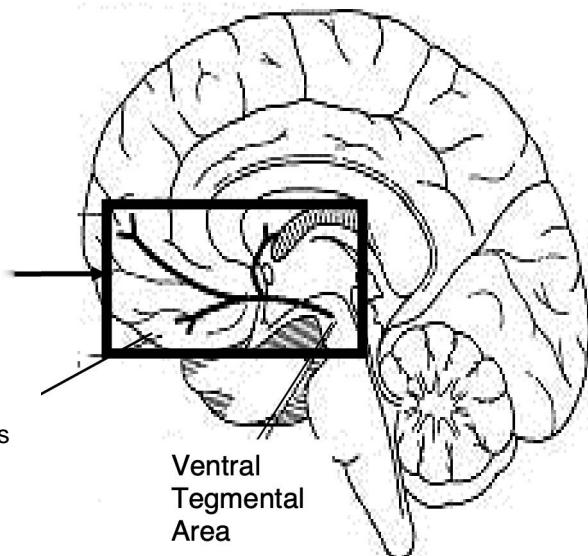


Figure 10 Schematic of the reward pathway involved in addiction. Figure modified from NIDA Notes, 1996.

The inhibitory action of dopamine has been shown to be enhanced in psychostimulant-sensitized animals and that this effect is also mediated by DI-like receptors (O'Donnell and Grace 1994). For example, GBR12909, a dopamine transporter (DAT) inhibitor, showed no effect on the synaptic action of dopamine following psychostimulant treatment indicating that the enhancement of the presynaptic inhibition is not likely to be attributed to downregulation of dopamine transporter activity (Beurrier and Malenka 2002). Rather, this change in presynaptic inhibition appears to be caused by a modification of the number of presynaptic DI -like receptors. Past results have shown, for instance, that chronic administration of the dopamine reuptake inhibitor cocaine results in increased inhibitory action of DI agonists on single-unit responses of nucleus accumbens neurons (Henry and White 1991). These data suggest that the effect of DI receptors in behavioral sensitization are the result of increased postsynaptic inhibition. This theory is supported by the decreased locomotor response and of

dopamine overflow following treatment with the D1 receptor antagonist SCH23390. The reduction of dopaminergic overflow in this study produced by SCH 23390 was not accompanied by a comparable increase in the dopamine metabolites DOPAC or HVA, indicating that the change occurs outside of the metabolism of dopamine and supports a change in the receptor functionality.

Although Vezina postulated that induction of behavioral sensitization requires the D1 but not the D2 receptor (1996), results here indicate a role of the D2 receptor in behavioral sensitization. However, this may be the result of differences in design and methodology. Vezina (1996) employed a pretreatment of SCH23390 for D1 antagonism or eticlopride, spiperone or sulpiride for D2 antagonism followed by amphetamine or saline treatment up to 1 hour later, and sensitization was tested by amphetamine challenge 1 -3 weeks later. In the present study, antagonist administration was followed by amphetamine administration by only 10 mins, that could be related the time course and action of eticlopride and its efficacy to block not only D2 subtypes but D3 receptor subtypes. Additionally, in the present study amphetamine was administered every other day for a total of seven exposures. In the Vezina (1996) study, amphetamine was administered at a dose of 1.0 mg/kg once every 3rd day for five injections. Though the doses of amphetamine, eticlopride, and SCH23390 were the same in both studies, it is likely that the differences in experimental design may account for the differences in effects at the D2-like receptors in these studies. It is not possible to exclude a possible impact of the D3 receptor since both quinpirole and eticlopride have possible effects at the D3 receptor in addition to the D2 receptors within this study. Vezina (1996) suggested that D2 receptors did not have a role in sensitization to amphetamine and

showed similar results using three dopamine D2 antagonists, two of that are more selective to the D2 receptor than eticlopride. Interestingly, eticlopride treatment to rats neonatally treated with saline resulted in locomotor activity levels that were similar to non D2-primed animals treated with amphetamine as seen in figures 2b and 3b. Dopamine D2-primed animals receiving eticlopride and amphetamine resulted in a locomotor profile very similar to that of non D2-primed subjects receiving eticlopride alone. Such findings suggest that systemic eticlopride can attenuate the locomotor effects of amphetamine, but that eticlopride treatment alone given in adulthood creates a change in dopamine receptors that results in behavioral changes similar to those seen from neonatal priming. Interestingly, past studies have shown that eticlopride (0.6 mg/kg) potentiates dopamine release by amphetamine using functional MRI as well as in locomotor activity (Tanabe et al. 2004; Chen YC et al. 2005), although in the latter study, eticlopride was infused into the VTA. There is no doubt that differences in administration routes (i.e. intrabrain administration versus systemic ip injection) and dosages may account for differential results, and, in fact, it suggests that the effects of systemic administration of eticlopride on dopamine overflow is fundamentally different from direct infuction into brain areas.

Interestingly, there were sex differences in the response to eticlopride. Pre-treatment with eticlopride blocked sensitization to amphetamine more effectively in females than in males but completely blocked amphetamine's effects on accumbal dopamine overflow equally across both sexes. This is inconsistent with our hypothesis but poses an interesting finding in that this is the only study to analyze the effects of

systemic treatment with the D2/D3 antagonist eticlopride on amphetamine sensitization and its effects on dopamine overflow.

Amphetamine administration significantly increased turning behavior in males and females, an effect that was blocked by both D1 and D2 antagonist treatment, though D1 blockade was more effective, similar to results with horizontal activity. This behavioral effect also may be the result of a greater density of striatal dopamine D1 receptors and turning behavior has been shown to be a valid test of striatal function.

Amphetamine action on neurotransmission is complex. First, amphetamine uptake by DAT is required. Second, amphetamine then inhibits the activity of vesicular monoamine transporter (VMAT) resulting in an increase in non-vesicular cytosolic neurotransmitter concentrations. This increase in neurotransmitter results in reverse transport of the neurotransmitter via membrane-bound transporters (Sulzer et al. 1993; Sulzer et al. 1995). Quantification of neurotransmitters by microdialysis following amphetamine administration is a result of overflow rather than vesicular release of the neurotransmitter. Results showed amphetamine-induced overflow of dopamine in the nucleus accumbens (core) in rats was greater in rodents repeatedly treated with quinpirole during the postnatal development period (Nowak et al. 2001).

It is hypothesized that the underlying mechanism of this effect is related to several factors. First, amphetamine produced a significant increase in dopamine overflow. Second, amphetamine has been shown to produce a subsensitization of presynaptic D2 autoreceptors in the VTA. Third, neonatal quinpirole produces supersensitization of postsynaptic D2 receptors resulting in an increased dopaminergic response to amphetamine (Nowak et al. 2001). This increased dopaminergic response

presumably results in a more robust behavioral activation to the drug. There is another complex caveat to this mechanism. Based on the fact that quinpirole is a D2/D3 agonist, it binds to and activates both the presynaptic inhibitory D2 autoreceptor as well as the postsynaptic D2 receptor. Past work has shown that acute quinpirole treatment actually produces a decrease in dopamine overflow (Nowak et al. 2001) in the dorsal striatum, and this is presumably due to its action at the autoreceptor. Therefore, it is assumed that quinpirole primes both the D2 inhibitory autoreceptor and the D2 postsynaptic receptor with roughly equal affinities. A critical action relative to the results observed here is that amphetamine produces subsensitivity of the inhibitory D2 autoreceptor. Therefore, supersensitization of the postsynaptic receptor as is produced by neonatal quinpirole is the most plausible explanation of the mechanism observed in the present study.

The mechanism of D2 receptor supersensitivity, or D2 priming, is unknown. When D2 receptors are activated, a G-protein coupled receptor (GPCR), like the dopamine-bound D2 dopamine receptor, binds a trimeric G-protein and catalyzes the exchange of GTP for GDP at the alpha subunit of the G-protein that leads to the dissociation of GTP bound alpha subunit. This dissociation allows the freed dimer to regulate activity of cellular effector molecules. Signal termination is mediated by the GTPase activity intrinsic to the alpha subunit. The GTPase hydrolyzes the bound GTP to GDP allowing the subunit to reassociate with the dimer (Ross and Wilkie 2000). GTPase activity of the alpha subunit and thereby the signal termination of the GPCR can be enhanced by members of a family of proteins called regulators of G-protein signaling (RGSs) (Berman and Gilman 1998, Ross and Wilkie 2000). One such protein,

RGS9, has a splice transcript RGS9-2 that is confined to the striatum (Thomas et al. 1998). Mice with a null mutation in the RGS9 gene showed heightened locomotor responses to cocaine and related psychostimulants, whereas viral-mediated overexpression of RGS9-2, but not RGS4, in rat striatum specifically reduced locomotor responses to D2 receptor agonists (Rahman et al. 2003). As mentioned above in the introduction, we have shown that RGS9 is significantly downregulated in D2 primed animals as compared to non D2-primed animals (Maple et al. 2007) in several heavily innervated dopaminergic brain areas, including the striatum, nucleus accumbens, and frontal cortex. Therefore, it appears that regulation of D2 receptor signal transduction is lessened in animals neonatally treated with quinpirole, likely resulting in an overall increased response from this family of receptors. Modifications of downstream proteins including, but not limited to, the RGS family of proteins may help to account for the modifications of the cascade resulting in greater or prolonged dopaminergic effect following amphetamine administration. However, it should be noted that no second messenger system has been identified to fully account for the supersensitization following neonatal quinpirole treatment.

Amphetamine has been shown to produce an additive effect on extraneuronal dopamine levels in both the striatum and nucleus accumbens core in rats neonatally treated with quinpirole (Nowak et al. 2001, Cope et al. 2009). This additive effect may result from amphetamine actions mediated by both the D1 and D2 receptors. In this study we attempted to elucidate the roles of each receptor. Results here suggest the D1 receptor does play a role in regulating the extraneuronal levels of dopamine in the nucleus accumbens core. However, eticlopride blockade of the D2 receptor also

attenuates the effects of amphetamine on extraneuronal dopamine in the nucleus accumbens. Such an impact of singular receptor blockade implicates mediation by synergistic effects of the D1 and D2 receptors. It has been shown that concomitant stimulation of the D1 and D2 receptors is required under normal conditions for many actions of dopamine, a phenomenon referred to as D1/D2 synergism (Gershnik et al. 1983, La Hoste et al. 1996). This type of receptor interaction is representative of an extreme form of synergism in that the effect of two combined drugs exceeds the algebraic sum of their two effects. In this form, two drugs (e.g. D1 and D2 agonists) potentiate the effects of the other and each is ineffective without the other based on the activation of both dopamine receptor subtypes. It is the concurrent activation of both receptor subtypes that results in the synergistic effects. In terms of amphetamine, an indirect dopamine agonist, administration results in an increased dopamine release resulting in stimulation of D1 and D2 receptors postsynaptically, an idea supported in work showing that blockade of presynaptic autoreceptors does not impact this D1 and D2 interaction (Shi et al. 1997).

Synergism is evident in the control of motor behavior. For example, reserpine-induced akinesia can be reversed by combined but not separate administration of a selective D1 or selective D2 agonist. Increases in locomotion and motor stereotypy elicited by the indirect dopamine agonists apomorphine and amphetamine can be blocked by either a selective D1 or D2 antagonist (Lewis et al. 1983, Martres et al. 1992). The results of this study provide further evidence that there is such interplay between dopamine receptors given the ability of either D1 or D2 antagonist treatment to prevent amphetamine-induced increases in locomotion. It must be noted that basal

levels of endogenous dopamine may provide sufficient D1 tone to synergize with an exogenous D2 agonist. Administration of a D1 agonist alone does not elicit motor stereotypy, whereas administration of a D2 agonist alone does (White et al. 1988).

Following selective destruction of central dopaminergic neurons with the neurotoxin, 6-hydroxy-dopamine (6-OHDA), or prolonged depletion of monoamines with reserpine, there is an increased expression of striatal D2 receptors and animals become supersensitive to D1 or D2 agonists (La Hoste et al. 1993a). Accompanying the agonist supersensitivity is a complete breakdown of requisite D1/D2 synergism. Functions that previously required concomitant D1/D2 stimulation can be mediated by independent stimulation of either D1 or D2 receptors. This is true for stereotyped motor behavior, inhibition of striatal neuron firing, and striatal c-fos expression (La Hoste et al. 1993b). D1/D2 synergism may then play a role in amphetamine induced behavioral sensitization but does not account for effects following D2 receptor priming as it is expected that such priming would break down the synergistic mechanism. A breakdown in D1/D2 synergism as a result of priming is thought to be the result of a lack c-fos expression in the striatum following D2 agonist treatment. The absence of c-fos causes a change in the downstream cascade of gene expression that persists for at least 8 hours, a time consistent with that of causal events leading to increased sensitivity and a breakdown in D1/D2 synergism (LaHoste et al. 1993b).

Functional modifications of dopamine receptors have been implicated to be critical in the process of sensitization with regard to psychostimulants including amphetamine, nicotine, and cocaine (Kalivas and Duffy 1993; Kalivas et al. 1993; Vanderschuren and Kalivas 2000). Such a functional change has been specified to

exist in the mesocorticolimbic pathway, the pathway implicated in behavioral sensitization (Kalivas et al. 1993).

Both dopamine D1 and D2-like receptors in the NAcc as well as glutamate NMDA and AMPA receptors located on medium spiny neurons in the VTA are primary targets for altered neurotransmission following amphetamine or cocaine treatment (Montague et al. 2004, Hyman et al. 2006). When animals are sensitized to a drug, prior research suggests, dopamine D1 receptor supersensitivity occurs in the nucleus accumbens as a result of the upregulation of the cAMP pathway (Nestler and Aghajanian 1997). The blockade of dopamine D1 receptors in the present study prevented amphetamine-induced sensitization, an effect that may be explained by changes in the cAMP pathway. This cellular event may impact downstream G-protein coupling and result in an upregulation of adenylyl cyclase and protein kinase A signals. D1 dopamine receptor-mediated cellular signaling cascade could then enhance the phosphorylation of the transcription factor cAMP response element binding protein (CREB) and the expression of immediate-early genes, such as Fos and Jun (Nestler 2002, Waters et al. 2003). Induction of expression of those genes is rapid and mostly transient and invokes subsequent gene expression (Zhang et al. 2005). For example, activation of CREB by phosphorylation is a common neuronal adaptation in the nucleus accumbens to psychostimulants (Impey et al. 2004). Previous studies suggest that drug-induced CREB activation/phosphorylation in the nucleus accumbens constitutes a negative feedback mechanism that dampens behavioral sensitivity to subsequent drug exposure (Carlezon et al. 2005).

Further, the accumulation of AFosB in the nucleus accumbens is a universal phenomenon following chronic exposure to abused drugs (Nestler et al. 1995). Over expression of AFosB in the nucleus accumbens increases the behavioral response to cocaine and amphetamine and the amount of AFosB accumulation in the nucleus determines the duration and intensity of drug-induced behavioral sensitization (McClung et al. 2004). Blockade of the D1 receptor with SCH23390 has been shown to attenuate the induction of fos proteins but D2 receptor antagonist treatment increased fos related proteins levels seen following amphetamine treatment (Nestler et al. 1995). In terms of the molecular mechanism underlying dopaminergic receptor involvement in behavioral sensitization, fos proteins, including AFosB, appear to play a larger role in changing the downstream gene expression following drug exposure. Results involving the fos proteins appear to indicate that the D1 receptor plays the mediating role in sensitization. Thus, modulation of dopamine receptors through antagonism may reduce the amphetamine-induced increases AFosB, allowing for a potential modulation of behavioral sensitization. A future study in this laboratory will analyze the expression of delta FosB within this model.

The results of this study support the theory that both D1 and D2 receptors have a role in amphetamine behavioral sensitization. Further, D2 primed animals appear to have an increased sensitivity at the D2 receptor such that the coupled treatment of quinpirole and eticlopride results in a stimulated response comparable to animals treated with amphetamine regardless of neonatal drug treatment. This may support the idea that D2 priming produces an autoreceptor subsensitivity that enhanced the dopaminergic response similar to that of amphetamine (Kostrzewska et al. 2008). As

expected, dopamine D1 antagonist treatment in D2-primed animals was more effective in reducing locomotor activity in female rats than male rats compared to saline treated control subjects because female rats are more susceptible to antagonist effects than males (Schindler and Carmona 2002). This supports previous work showing that females are more susceptible to the effects of dopamine D1 receptor blockade than are males, although D2 blockade using eticlopride has been shown to be effective at blocking cocaine sensitization in at least one past study (Schindler and Carmona 2002), whereas in the present study eticlopride was not effective at blocking amphetamine sensitization in adult females. This points to a possible sexual dimorphism not only in the response to antagonists but in the combination of dopaminergic antagonists with amphetamine.

With regard to D2 receptors, increased antagonist susceptibility may arise from dopaminergic fluctuations related to estrogen levels and stages of the estrus cycle. In females, estrogen acts to inhibit GABA neurons in the striatum and accumbens resulting in increased dopamine function. Estrogen also acts to enhance dopamine release by downregulating D2 receptor function (Becker 1999). Though no effects of estrus cycle stage were found associated with sex differences in locomotor activity in the present study, that may be the result of insufficient sample size in each stage of estrus. However, animals were treated every other day for the duration of sensitization; therefore, female subjects should have been administered adult drug treatment and antagonist across all stages of the cycle.

D2 primed animals treated with eticlopride exhibited no difference relative to saline treated controls on the horizontal activity measure. However, non D2-primed

females treated with eticlopride and saline exhibited an increase in locomotor activity as compared to saline- treated control subjects. This is likely the result of presynaptic autoreceptor blockade that results in a disinhibition of dopamine release, thereby stimulating additional dopamine release and increasing locomotor activity (Goldwert et al. 2006). Female rats showed a greater reduction in activity following SCH23390 treatment as compared to males, consistent with past work (Schindler and Carmona 2002). This difference in DI response may also be the result of dopaminergic changes associated with estrogen (Becker 1999). However, the nucleus accumbens plays a significant role in behavioral sensitization and males show a higher density of DI receptors in the nucleus accumbens (Andersen et al. 1997). This difference in receptor density may also account for an increased impact in females. Less dense populations of receptors would likely result in a greater occupancy of receptors by a lower dose of antagonist and may account for females responding in a more significant way to receptor blockade. As expected, males showed a less robust sensitization response to amphetamine compared to females. This expectation was based on previous work that has shown that the DI receptor plays a more important role in amphetamine sensitization, although there are no data on sex differences in the roles of DI and D2 receptors in amphetamine sensitization.

In terms of behavioral sensitization, past work has shown that females exhibit more robust sensitization than do intact males to amphetamine (Camp and Robinson 1988, Becker 1999, Perna et al. 2007, Cope et al. 2008). We hypothesized that female rats would be more sensitive to the antagonism treatments of both DI and D2 receptors. One of the more important and interesting effects reported here was that

blockade of the D1 receptor completely blocked sensitization to amphetamine in both males and females, at least implying D1 receptor function is similar across the two sexes in terms of amphetamine sensitization. In contrast, blockade of the D2 receptor by eticlopride was more effective at blocking amphetamine sensitization in males as compared to females, suggesting a sex difference in D2 function. One issue here is that eticlopride is only 3-4 fold more selective for the D2 receptor subtype as compared to the D3 receptor subtype (Claytor et al. 2006). Thus, this may be reflective of sex differences of not only the D2 but D3 receptor subtype of the D2 receptor family. A more selective D2 receptor antagonist may be able to more effectively ascertain the roles of the D2 and D3 receptors in males and females. In other work from this lab, both D2 and D3 receptor subtypes have been shown to be involved in the locomotor activating effects of nicotine (Sheppard 2008). A second issue here, however, is that sex differences in D2 receptor function have been shown. Females demonstrate a higher amount of locomotor activation when D2 receptors are activated by an agonist (Szumlinski et al. 2000, Schindler & Carmona, 2002) but locomotor depression to D2 antagonism, although this study used adolescents (Sheppard et al. in press). Additionally, age differences in the development of dopamine receptors prevalent in the striatum and nucleus accumbens have been shown (Andersen & Teicher, 2000). Females demonstrate a more stable number of D2 receptors throughout development, whereas males exhibit a lower amount of D2 receptors in early adolescence with the number peaking in mid-adolescence before being reduced to equivalent numbers of D2 receptors as females in adulthood. This may have repercussions as to the adult dopamine function as well, but this has yet to be delineated. Regardless, these results

support past work that sex differences in D2 function and D2 receptor development exist.

There are no results from DOPAC or HVA samples that indicate increased dopaminergic overflow is the result of a change in dopamine metabolism. D2-primed animals treated with saline as well as primed animals treated with SCH23390 and amphetamine revealed a significantly higher level of DOPAC in microdialysate samples as compared to saline treated controls. These results suggest that the D2 priming related increase in DOPAC is not related to D1/D2 synergism, as said increase can not be fully attenuated following D1 receptor blockade. Additionally, at 40 minutes post drug treatment, non-D2-primed animals treated with SCH23390 show elevations in DOPAC release. This may indicate an increase in dopamine catabolism that resolves at 60 minutes potentially indicating a freeing up of previously bound receptors or a self-resolving mechanism following dopamine catabolism. This follows the results that SCH23390 can increase dopamine overflow due to its action on presynaptically located D1 receptors (Egilmez et al. 1995). Thus, it is then likely that changes occur within presynaptic receptor functionality rather than dopamine metabolism following release.

Maybe the most important aspect of the present study is that these results are consistent with clinical findings. Schizophrenic patients, using a single photon emission computerized tomography (SPECT), have shown increased striatal dopamine release in response to an amphetamine challenge as compared to healthy matched control subjects (Laruelle et al. 1996, Abi-Dargham et al. 1998). Increased dopaminergic transmission was also associated with behavioral consequences (e.g. worsening or emergence of psychotic symptoms). The effects of amphetamine on subjects were not

attributable to peripheral effects of the drug. More specifically, increased striatal dopamine release following amphetamine administration is associated with the D2 receptor sensitivity (Laruelle et al. 1996, Abi-Dargham et al. 1998). These studies present a clinical correlation for the findings of this study in terms of increased dopaminergic overflow and locomotor findings. Taken together, these results confirm the involvement of the dopamine D2 receptor in terms of modeling psychosis, and more specifically, schizophrenia.

In the current study, amphetamine was shown to significantly increase norepinephrine overflow in both D2-primed and non D2-primed male and female rats, although neonatal quinpirole treatment did not enhance this effect. This result is consistent with work by McKittrick and Abercrombie (2007) who have shown systemic amphetamine administration resulted in a significant increase in norepinephrine overflow in the nucleus accumbens. Treatment with either D1 or D2 antagonist blocked the amphetamine-induced increase in norepinephrine overflow in the nucleus accumbens.

It has been shown that norepinephrine overflow in the rat nucleus accumbens is under the opposing influence of stimulatory dopamine D1 and inhibitory dopamine D2 receptors (Vanderschuren et al. 1999). The D2 antagonist sulpiride has been shown to strongly increase norepinephrine overflow in the nucleus accumbens, whereas the D1 antagonist SCH23390 did not reduce norepinephrine overflow (Vanderschuren et al. 1999). Results of this study similarly show that SCH23390 does not significantly reduce norepinephrine as compared to control subjects; however, the D2-like antagonist eticlopride does not appear to increase norepinephrine overflow. Inhibitory dopamine

D2 receptors have been shown to regulate norepinephrine overflow, as opposed to the enhancing effects of the dopamine D1 receptor in an in vitro study using superfusion techniques (Vanderschuren et al. 1999). Results here support that idea as eticlopride as a D2 like antagonist blocked norepinephrine overflow in the nucleus accumbens. It is important to note that effects of eticlopride may not be limited to the D2 receptor, as eticlopride also has significant affinity for the D3 receptor. The limitations of this study do not allow a complete understanding of any potential involvement at D3 receptors.

The combined treatment of quinpirole and amphetamine result in an increased norepinephrine overflow at the 80, 100, and 120 minute time points. If released endogenous dopamine tonically inhibits norepinephrine overflow in the nucleus accumbens through the stimulation of D2 receptors, then D2 primed animals, as well as non D2-primed animals treated with amphetamine should have similar norepinephrine levels to saline controls an idea supported by the data. Dopamine D2 receptors are located near active zones formed by dopamine and norepinephrine terminals, whereas dopamine D1 receptors are located more distally from the site for dopamine overflow (Sesack et al. 1994, Hersche et al. 1995). It may be that dopamine released from mesolimbic neurons preferentially interacts with D2 receptors located closer to the terminal of release (Vanderschuren 1999). If such a proximal relationship prevails, blockade of the D2 receptor by eticlopride would then inhibit norepinephrine overflow in the nucleus accumbens. Dopamine D2-primed animals treated with amphetamine as a result of the increased dopamine overflow would activate both D1 and D2 receptors resulting in a net increase in norepinephrine overflow. Dopamine D2 receptor priming

alone would not be expected to enhance norepinephrine overflow but rather to inhibit such overflow.

### Conclusions

Dopaminergic dysfunction is implicated in underlying several mental illnesses including schizophrenia, obsessive-compulsive disorder, and bipolar disorder. Mental illnesses are often comorbid with substance abuse disorder leading to a dual diagnosis. Such a dual diagnosis complicates treatment and may impact the overall effectiveness of pharmacological interventions. Using a D2 priming model to mimic dopamine D2 receptor dysfunction in mental illness, changes in receptor sensitivity result in changes in amphetamine-induced locomotor response and dopamine overflow in the nucleus accumbens. Results of this study are that D2 priming enhanced the dopaminergic response to amphetamine in adulthood, an effect alleviated by blockade of either D1 or D2 receptors. Second, changes in D2 receptor sensitivity may increase the positive reinforcing effect of a psychostimulant drug in adult animals. Third, D1 receptor blockade was more effective in reducing locomotor activity in males and dopamine overflow than D2 receptor blockade by eticlopride. Fourth, females are more sensitive to the effects of D1 receptor blockade than males.

If supersensitization is a key aspect of psychosis, these changes may represent a mechanism toward the increased abuse of drugs in humans suffering from these psychotic disorders. Blockade of dopamine D2 receptors results in patterns of locomotor activity and dopaminergic overflow similar to that seen from amphetamine administration, likely a result of presynaptic autoreceptor inhibition. Dopamine D1 and D2 receptors differentially play a role in amphetamine-induced sensitization. Dopamine

DI receptor blockade is more effective at preventing locomotor sensitization than D2 receptor blockade. This may indicate a primarily motor involvement of the DI receptor family in terms of behavioral sensitization rather than involvement in the rewarding properties of amphetamine. Conversely, dopamine D2 receptor may play less of a role in the activating effects of amphetamine, but when antagonist to this receptor are given systemically, they appear to produce a different effect on dopamine function than when infused. Precisely the mechanism behind this is not known and outside of the scope of the present work but opens up an interesting area of research. All antipsychotic drugs antagonize the D2 receptor (Tollefson 1996) and are obviously given systemically. Thus, it appears that the route in that this drug is given may have important implications relative to its effects on neurochemistry and specifically, dopamine function.

It is unclear what impact sex differences have in this interaction. Females were more susceptible to the effects of dopamine antagonists than males. Further experimentation is required to clearly elucidate sex differences in D2 primed animals and how this may be related to hormonal fluctuations. Clinical results have shown that female patients are more sensitive to the effects of abused drugs (Carroll et al. 2004), a result supported here. Many such measurements of response to a drug in a clinical setting are subjective and cannot be modeled in animals. Animal models are then limited in allowing a full understanding of underlying sex differences in a dual diagnosis. Dopaminergic dysfunction may underlie mental illnesses and increases in dopamine D2 sensitivity may represent a mechanism for vulnerability to psychostimulant abuse, particularly in terms of comorbidity.

Substance abuse in schizophrenics carries consequences that further impact schizophrenic patients. Results of the current study correlate with clinical findings that amphetamine increases dopaminergic transmission in schizophrenic patients, and that increases in dopaminergic transmission are associated with a worsening of behavioral symptoms of schizophrenia. This model may then allow for further exploration of mechanism(s) that result in a greater incidence of drug use, particularly psychostimulants, in schizophrenics compared to the general population.

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