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Amphetamine Sensitization and \textit{in vivo} Microdialysis of the Nucleus Accumbens Core of Adult Male and Female Rats D2-Primed as Neonates.

Zackary Adam Cope
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Amphetamine Sensitization and in vivo Microdialysis of the Nucleus Accumbens Core of Adult Male and Female Rats D2-Primed as Neonates

A thesis
presented to
the faculty of the Department of Psychology
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Arts in Psychology

by
Zackary A. Cope
August 2008

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ABSTRACT

Amphetamine Sensitization and in vivo Microdialysis of the Nucleus Accumbens Core of Adult Male and Female Rats D2-Primed as Neonates

by

Zackary A. Cope

Neonatal administration of quinpirole produces significant increases in D2 receptor sensitivity that persists into adulthood. This phenomenon, known as D2 receptor priming, is consistent with pathology in schizophrenia. Rats were administered quinpirole or saline postnatally and raised to adulthood. In adulthood, rats were administered d-amphetamine sulfate or saline every other day and were placed in a locomotor arena where activity was measured over 7 trials. Results showed that D2-primed rats receiving amphetamine were higher in locomotor activity across all days of testing compared to other groups. This effect was more prominent in males than in females. After sensitization, cerebrospinal fluid was taken via microdialysis from the nucleus accumbens core and was analyzed for dopamine content. Analysis revealed D2 priming produced a 300% increase of dopamine release in the nucleus accumbens core in response to amphetamine compared to controls. These results suggest that increases in D2 sensitivity may lead to increased reaction to amphetamine in psychotic individuals.
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Individuals afflicted with schizophrenia exhibit a range of behavioral malfunction including, but not limited to paranoia, disruption and disorganization of regular thought patterns, inappropriate perception, hallucinations, and flattened affect (Spitzer, Weisbrod, Winkler, & Maier, 1997; Wilson, Sanyal, & Van Tol, 1998). The schizophrenic population also abuses psychostimulants at a significantly higher rate than the general population (LeDuc & Mittleman, 1995). Current estimates suggest the incidence of psychostimulant abuse by schizophrenics to be anywhere from 55%-85% (Sacco et al., 2005). When compared to the general population, which demonstrates a 7.5% rate of abuse (LeDuc & Mittleman), the incidence of psychostimulant abuse is found to be between 7-12 times higher in the schizophrenic population. The prevalence of psychostimulant abuse is also much higher among schizophrenics when compared to populations with other mental health diagnoses, which may indicate a tendency towards drug abuse resulting from the specific pathology of the disease itself (Richard, Liskow, & Perry, 1985). This interpretation is strengthened by subsequent research suggesting that schizophrenics preferentially abuse drugs with stimulant properties rather than drugs with non-stimulant properties such as alcohol, opiates, or barbiturates (Dixon, Haas, Weiden, Sweeney, & Frances, 1990; Schneier & Siris, 1987; Zemishlany, Aizenberg, Weiner, & Weizman, 1996).

Clinical Consequences of Substance Abuse in Schizophrenia

Psychostimulant abusing individuals suffering from schizophrenia face more severe consequences when compared to nonabusing schizophrenics. Most notable is an increase of the positive and negative symptoms inherent in the disease pathology namely psychosis, anhedonia, and cognitive impairment (Jeste, Gladsjo, Lindamer, & Lacro, 1996). In addition, schizophrenic
abusers of psychostimulants demonstrate a much higher incidence of suicidal ideation with associated depression upon admission to the hospital (Seibyl et al.). It has been demonstrated that substance-abusing schizophrenics tend towards more hospitalizations over the course of their lifetime compared to those reporting no substance abuse (Brady, Casto, Lydiard, Malcolm, & Arana, 1991). The symptoms of schizophrenic patients lacking an accurate medical history of drug abuse are often misclassified and mistreated by health professionals, leading to a much poorer course of treatment and subsequent exacerbation of disease psychopathology (Seibyl et al.). This outcome is likely due to the findings that self-administration of psychostimulant drugs worsening psychotic symptoms that persist for longer than the half-life of the drug (Brunette, Mueser, Xie, & Drake, 1997; Dervaux et al., 2001). This may be the result of a persistent dysregulation of mesolimbic dopamine repeatedly observed in psychostimulant abuse (Koob et al., 2004) affecting the already increased dopamine concentrations of schizophrenic individuals (Heinz, 2000).

Gender Differences in Schizophrenia

Gender differences play a significant role in determining the course and symptoms manifested in schizophrenia. Currently, the most clearly illustrated differences are the age of the individual’s first schizophrenic episode (Szymanski et al., 1995) and the severity of impairment as related to overall functioning (Goldstein, 1988; Goldstein & Link, 1988; McGlashan & Bardenstein, 1990). Specifically, schizophrenic women demonstrate a later age of onset (Szymanski et al.) and much higher capacity for overall functioning than schizophrenic men (Goldstein & Link, ; McGlashan & Bardenstein). Women also demonstrate increased responsiveness to neuroleptic medication used to treat the disease (Szymanski et al.), which is congruent with the finding that schizophrenic women require less frequent hospitalization, and
they are hospitalized for shorter periods of time (Goldstein). Women are also less vulnerable to paranoia and hallucinations while demonstrating a higher ability for interpersonal psychosocial functioning than men (Andia et al., 1995) who demonstrate more negative symptoms such as flattened affect and cognitive deficits of greater severity compared to affected women (Szymanski et al.).

Thus far, it is unclear if gender differences play as strong of a role in the interaction between schizophrenia and habitual drug abuse. Initial studies have indicated that substance abusing schizophrenic women lose their capacity for higher functioning and become statistically identical to abusing schizophrenic males as indicated by similar scores on the Global Assessment of Functioning (Gearon & Bellack, 2000). In accord with clinical observations, there is also experimental evidence to suggest that schizophrenic women may be differentially vulnerable to negative effects of substance abuse (LeDuc & Mittleman, 1995).

A Rodent Model of Schizophrenia

Animal models have been useful in developing numerous experimental treatments while minimizing the risk of implementing these treatments in a clinical setting. However, due to the complexity and limited understanding of the etiologies of mental illnesses, appropriate animal models have been difficult to develop. But, as new light shed is on these disorders, researchers have been able to accurately model diseases such as schizophrenia bringing about disease specific behavioral abnormalities by inducing specific neurological abnormalities found in individuals with the disorder. Still, it remains important that these induced neurological abnormalities be similar to those found in the clinical population as to allow for the development of realistic treatments.
Acute drug induced model of schizophrenia. In schizophrenia, hyperactivity of the dopamine neurotransmitter system has been widely proposed as the neurochemical culprit. Several rodent models have attempted to model dopaminergic hyperactivity via pharmacological intervention. One approach has been to administer a drug known to produce large increases in dopamine such as phencyclidine (PCP) or cocaine and then behaviorally test the animal while it is under the influence of these drugs (Lacroix, Broersen, Feldon, & Weiner, 2000; Tilson & Rech, 1973). These models, although useful in demonstrating the effects of acute drug administration and subsequent hyperactivity in the dopamine system, fail to produce long-term dopamine hyperactivity and, thus, fail to provide a model of the developmental aspects of the disease. On the other hand, these models led to some insights on the underlying mechanisms in schizophrenia. For example, findings using the acute drug induced model have shown that dopaminergic dysregulation in the medial prefrontal cortex plays a role in modulating sensory gating, a function that is impaired in human schizophrenics as well as in many animal models of schizophrenia (Lacroix et al.).

Neonatal ventral hippocampal lesion model. A different rodent model of schizophrenia capitalizes on the decreases in synaptic connectivity in the hippocampus known to be present in schizophrenia (Lipska & Weinberger, 2000). In this model, the ventral portion of the hippocampus is ablated. The hippocampus is a region of the brain known to play a major role in cognitive performance. In the neonatal ventral hippocampal lesion (NVHL) model, 7-day old rats are administered a lesion of the hippocampus in an attempt to mimic hippocampal neuropathology known to exist in schizophrenia. This model has been used to effectively test the social, behavioral, and cognitive impairments known to exist in schizophrenia such as increased
anxiety, social impairment, isolation, and inhibition of the startle response to a repeatedly cued frightful stimulus (Sams-Dodd, Lipska, & Weinberger, 1997).

Although this model has shown several consistencies with the disorder, it remains that there has been no definitive evidence to suggest incidence of cell death in the hippocampus of schizophrenics (Harrison, 2004; Harrison & Eastwood, 2001) despite evidence of extensive anomalies in the synaptic development of hippocampal neurons (Harrison, 1999). Also, it remains to be determined as to what extent other regions of the brain may be damaged in producing this model of schizophrenia as well as whether or not the behavioral deficits witnessed might be due to collateral damage in other brain regions.

Disrupted in schizophrenia 1 transgenic mouse model. Newly developed transgenic mice models could be valuable thanks to their ability to model mental illnesses at the genetic and developmental level where many mental illnesses are thought to originate. The Disrupted in Schizophrenia 1 (DISC1) protein knockout or transgenic mouse model manipulates the expression or functionality of a genetic transcript that has been repeatedly found disrupted in individuals with schizophrenia or related disorders such as schizoaffective disorder, bipolar disorder, and recurrent major depression (Millar, Wilson-Annan, et al., 2000). The DISC1 protein disruption, only recently reported in 2000, is thought to affect genetic expression in multiple locations such as the heart, kidneys, testis, and placenta but maintains the highest transcript concentrations in the brain, specifically in the dentate gyrus and CA1-CA3 regions of the hippocampus (Millar, Christie, Semple, & Porteous, 2000). The DISC1 mouse model of schizophrenia shares multiple anatomical anomalies repeatedly demonstrated in human schizophrenics, most notably decreased connectivity in the hippocampus without cell death as well as enlarged lateral ventricles. Behaviorally, DISC1 disrupted mice demonstrate some
clinically relevant correlates, such as small disturbances in sensory gating (Hikida et al., 2007) and impairment of working memory in a match-to-place task (Koike, Arguello, Kvajo, Karayiorgou, & Gogos, 2006).

This model demonstrates a potentially useful new approach to the study of schizophrenia. However, it must be noted that this model is relatively new and untested. For instance, although multiple anatomical correlates exist between the model and the clinical population, it is, as of yet, unreported to what extent other anomalies exist in the brain tissue of DISC1 knockouts and mutants. Also, although deficits in sensory gating are reported, it must be noted that published reports of such deficits are not nearly as robust as seen in other models as they were limited to one level of the prepulse and were not significant on any other level of testing (Hikida et al.). However, reported unpublished data may report more robust PPI deficits that have been demonstrated in other more prevalent models (Ishizuka, Paek, Kamiya, & Sawa, 2006).

D2 receptor priming model. The abnormal behaviors observed in schizophrenia relate to increased activity of the dopamine D2 receptor (Crow, 1979; Davis, Kahn, Ko, & Davidson, 1991). This effect is akin to the action of drugs that produce similar symptoms in humans (Castaneda, Becker, & Robinson, 1988; Kokkinidis & Anisman, 1980). All antipsychotic drugs used to treat schizophrenia block the D2 receptor with some affinity (Tollefson, 1996). The drug quinpirole, a drug that serves as an agonist to D2/D3 receptors, has been shown to produce hyperactivity of the D2 receptor when administered to rats postnatally between days 1-11, 1-21, or 21-35, and this increase in D2 sensitivity has been shown to persist throughout the animal's lifetime. This phenomenon is known as 'D2 priming' (Kostrzewa, 1995; Kostrzewa, Brus, Rykaczewska, & Plech, 1993; Kostrzewa, Guo, & Kostrzewa, 1993). The neurochemical pathology of schizophrenia, however, does appear to be more complex than just changes in the
D2 receptor and involves several other neurotransmitter systems such as acetylcholine, serotonin, and glutamate (Harrison, 1999; Iqbal & van Praag, 1995; Lipska & Weinberger, 2000). Additionally, schizophrenics demonstrate significant increases in glucocorticoid hormones (Cotter & Pariante, 2002).

Regardless of the model’s focus on a single neurotransmitter system, there are several consistencies of the D2 priming model with behavioral deficits in schizophrenia. First, studies using Positron Emission Tomography (PET) have illustrated robust increases of dopamine release in the striatum of human schizophrenics exposed to amphetamine (Abi-Dargham, 1999) or methamphetamine (Lavalaye et al., 2001). Consistent with these results, MRI and Magnetic Resonance Spectroscopy (MRS) results have demonstrated increased D2 receptor density (Soares & Innis, 1999). These effects have been mimicked in the D2 primed rat using in vivo microdialysis, a technique that involves taking measurements of cerebrospinal fluid from the striatum of a live rat. Results have shown that D2 primed rats experience a 5-fold increase of dopamine in the dorsal striatum when administered an acute injection of d-amphetamine using the microdialysis technique, which is similar to past findings in schizophrenics (Nowak, Brus, & Kostrzewa, 2001).

Priming of the D2 receptor through neonatal treatment with quinpirole in the rodent has also been shown to be at least somewhat consistent with deficits in sensorimotor gating in schizophrenics. Sensorimotor gating can be measured through a behavioral task called prepulse inhibition (PPI). In this test, the subject is presented with a nonstartling auditory stimulus (known as the prepulse) that is followed shortly after by a much louder auditory stimulus (known as the pulse) intended to startle the animal or subject. The prepulse should come to predict the pulse, and thus, the animal should demonstrate an inhibition in the startle response when the prepulse is
administered. Research has shown that schizophrenics demonstrate approximately a 50% decrease in prepulse inhibition (Braff, Geyer, & Swerdlow, 2001). In our laboratory, we have shown that D2-primed rats demonstrate approximately a 20%-25% decrease in prepulse inhibition depending on the auditory intensity of the prepulse (Maple et al. manuscript in preparation). Although this is somewhat inconsistent with findings in schizophrenics, it remains that D2-primed rats demonstrate a significant PPI deficit, and it could be that increases in sensitivity of the D2 receptor contribute approximately half of this deficit, with the other half accounted for by other changes in brain function such as changes in acetylcholine (Crook, Tomaskovic-Crook, Copolov, & Dean, 2001; Leonard et al., 2002), glutamatergic (Harrison, 2004), or serotonergic systems (Iqbal & van Praag, 1995). Current research has repeatedly demonstrated that activation of the D2 receptor produces subjects that are significantly slower than normal controls in their ability acquire sensory gating (Kumari & Sharma, 2002), and it appears that the D2 receptor plays a more important role in PPI as compared to the D1 receptor (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001).

Several studies have reported severe cognitive deficits in schizophrenia such as impaired sustained attention or vigilance (Green, 1996), dysfunction of verbal (Fleming, Goldberg, Gold, & Weinberger, 1995) and visio-spatial (Keefe, Lees-Roitman, & Dupre, 1997; Park & Holzman, 1992) working memory, verbal learning (Green, Kern, Braff, & Mintz, 2000), problem solving (Bustini et al., 1999), and social cognition (Silver & Shlomo, 2001). Occurrence of these deficits are so consistently manifested that many posit them as the core symptoms of schizophrenia (Elvevag, Egan, & Goldberg, 2000).

Using the D2 priming model, similar cognitive deficits have been demonstrated in rats (Brown et al., 2002, 2004, 2005; Thacker et al., 2006). Specifically, these studies have
demonstrated deficits in spatial memory as demonstrated by differential performance on the Morris Water Maze (MWM) task as compared to controls. In this task, animals are placed in a pool of black colored water and there is a small platform hidden located slightly beneath the surface of the water that allows the rat to exit the aversive water environment. After multiple training trials, saline treated controls repeatedly outperform D2 primed rats in on the probe trial given at the end of acquisition as well as on the working memory version of the task, known as the match-to-place version (Brown, Flanigan et al., 2004; Brown, Gass, & Kostrzewa, 2002; Brown, Thompson et al., 2004).

The evidence for D2 priming as a rodent model of schizophrenia is bolstered by the findings that drugs intended to alleviate cognitive deficits in human schizophrenics by blocking D2 hyperactivity also produce similar effects in the D2 primed rat. More recent work has shown that the atypical antipsychotic olanzapine alleviated the spatial memory deficits of D2 primed rats. Additionally, olanzapine also eliminated the significant increase in yawning produced by D2 priming with quinpirole, essentially reversing D2 hyperactivity (Thacker et al., 2006). Finally, olanzapine was also shown to significantly alleviate significant decreases in neurotrophic factors in the hippocampus produced by priming of the D2 receptor, suggesting a significant effect of this drug on neuroplasticity in the hippocampus that may be underlying these deficits. Therefore, it appears that antipsychotic drugs are effective at alleviating behavioral deficits and changes in neurochemistry related to brain plasiticity produced by priming of the D2 receptor.

Finally, there are also data to demonstrate the presence of numerous neurochemical anomalies in D2 primed rodents that are also present in human schizophrenics. First, D2 priming has been shown to produce a 36% decrease in choline acetyltransferase (ChAT) as well as a
significant decrease in nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the hippocampus compared to saline controls (Brown, Flanigan et al., 2004; Brown, Thompson et al., 2004). These results agree with research that has indicated that nerve growth factor is also significantly decreased in nonmedicated human schizophrenics, which has been suggested to account for the multiple neurodevelopmental abnormalities caused by the disease (Aloe, Iannitelli, Angelucci, Bersani, & Fiore, 2000; Parikh, Evans, Khan, & Mahadik, 2003). The former findings of decreased ChAT from D2-primed rodents may also demonstrate congruence with human studies in that human schizophrenics exhibit decreases in density of the alpha4beta2 nicotinic receptor in the hippocampus (Durany et al., 2001), the alpha7 nicotinic receptor subunit gene (Leonard et al., 2002), and the muscarinic receptor (Raedler et al., 2003), which may be due to down regulation of these receptors when acetylcholine synthesis is reduced.

An Animal Model of Substance Abuse: Sensitization

The term behavioral sensitization is a learning phenomenon defined as increases in responding to repeated presentations of a stimulus. In drug abuse, behavioral sensitization is thought to underlie mechanisms of addiction (Robinson & Berridge, 1993) and is typically manifest as an augmented behavioral response following repeated presentations of the drug (Kalivas, Pierce, Cornish, & Sorg, 1998). Research has shown that modifications in dopamine function is critical for sensitization to several psychostimulants including amphetamine, nicotine, and cocaine (Kalivas & Duffy, 1993; Kalivas, Sorg, & Hooks, 1993; Vanderschuren & Kalivas, 2000). Specifically, increased in dopaminergic function in the mesocorticolimbic pathway has been hypothesized to play a significant role in behavioral sensitization (Kalivas et al., 1993).

The mesocorticolimbic pathway begins in the ventral tegmental area of the midbrain from which it projects to the prefrontal cortex and the ventral region of the basal ganglia. Structures
receiving connections from the latter region of the pathway, the nucleus accumbens core and the ventral striatum, appear to play significant roles in induction of behavioral sensitization (Kalivas et al., 1993). Studies have demonstrated increased dopamine function in these areas in vivo and *in vitro* following repeated administrations of d-amphetamine (Castaneda et al., 1988; Kolta, Shreve, De Souza, & Uretsky, 1985) as well as localized infusions of d-amphetamine into the nucleus accumbens of previously sensitized animals (Pierce & Kalivas, 1995). Previous microdialysis studies investigating the effect of d-amphetamine have demonstrated a two-fold increase in dopamine concentration in the nucleus accumbens core when compared to the shell following acute administrations of the drug (McKittrick & Abercrombie, 2007), a structure thought to underlie reinforcement and learning. This result is consistent with findings from self-administration studies, which have likewise demonstrated significant increases in extracellular dopamine levels in the nucleus accumbens following self-administration of d-amphetamine (Di Ciano et al., 1995).

The nucleus accumbens alone, however, is not solely responsible for sensitization on the cellular level. It has been demonstrated that the actions of dopamine cell bodies in the ventral tegmental area are necessary and sufficient for amphetamine sensitization while actions of dopamine cell bodies in the nucleus accumbens are not sufficient to produce an increased cellular response for locomotor sensitization. However, the nucleus accumbens is necessary for the behavioral induction, or expression of sensitization (Cador, Bjijou, & Stinus, 1995; Hooks, Jones, Liem, & Justice, 1992), demonstrating the importance of this area in amphetamine-induced behavioral sensitization.
Dopamine Receptors: Their Involvement in Behavioral Sensitization to Psychostimulants

Two dopamine receptor families have been identified in the central nervous system. The D1-like family includes the D1 and D5 subtypes, whereas the D2-like family includes the D2, D3, and D4 subtypes. Both families of receptor are slow-acting, metabotropic receptors, meaning binding of these receptors initiates a G-protein-mediated biochemical cascade that, in turn, affects cellular communication. This class of receptor contrasts the actions of fast synaptic or ionotropic receptors that immediately change their conformation upon binding of the appropriate ligand (Girault & Greengard, 2004).

Although data have determined the importance of dopamine to behavioral sensitization (Vezina & Queen, 2000; Vezina & Stewart, 1989), it remains unclear as to exactly which receptor family plays the larger role. According to the majority of evidence, the D1 receptor seems to play a bigger role in behavioral locomotor sensitization to amphetamine than the D2 receptor, an effect that has been demonstrated by attenuation of amphetamine sensitization via blockade of the D1 receptor (Drew & Glick, 1990). Concurrently, D1 receptor knockout mice failed to exhibit sensitization to amphetamine (Karper et al., 2002). Additionally, blockade of the D1 receptor by the D1 receptor antagonist SCH-23390 blocks sensitization to amphetamine while other antagonists known to have a greater affinity for the D2 receptor subtypes do not produce similar results (Vezina, 1996). Signaling by the D1 receptor subtype has been shown to be directly altered during behavioral sensitization to amphetamine evidenced by its increased sensitivity to amphetamine as measured by electrophysiological recordings as well as the differential genetic expression that is dependent upon induction by the D1 receptor, specifically Fos, FosB, and JunB (Hu et al., 2002).
However, pharmacological blockade of two D2 receptor subtypes, specifically the D3 and D4, have also been shown to attenuate sensitization and dopamine release (Chiang, Chen, & Chen, 2003; Feldpausch et al., 1998), but these effects appear to be much more secondary to the role of the D1 receptor. For example, studies have demonstrated that high doses of the D2 receptor antagonist eticlopride are sufficient to block locomotor sensitization, whereas pre-exposure to low doses of eticlopride actually produced enhanced activity following exposure to amphetamine. This effect could be due to elevation of extracellular dopamine levels in the ventral tegmental area induced by low dose eticlopride (Tanabe, Suto, Creekmore, Steinmiller, & Vezina, 2004). It has also been hypothesized that the D3 receptor subtype may play a role in inhibiting locomotor induction, but increased and repeated binding of these receptors may, in turn, produce tolerance of these effects in the cell. So, as the D3 receptor becomes tolerant to amphetamine, less inhibitory effects are produced, which may indirectly induce locomotor sensitization (Richtand et al., 2000).

**Sex Differences and the Estrus Cycle and its Relationship to Psychostimulant Sensitization**

There are considerable data to suggest that sex plays a substantial role in differential biological responses to psychostimulants in sensitization of psychomotor behavior (Becker, 1999; Bowman et al., 1999; van Haaren & Meyer, 1991). Female rats chronically exposed to amphetamine or nicotine exhibit significantly greater locomotor activity when compared to comparably exposed males (Becker; Booze, Wood, Welch, Berry, & Mactutus, 1999; Melnick & Dow-Edwards, 2001). Additionally, female rats have been shown to demonstrate a significantly greater stereotypical behavior such as yawning and chewing (Beatty & Holzer, 1978) as well as rotational behaviors compared to males when receiving amphetamine (Robinson, Becker, & Ramirez, 1980).
It has been suggested that sex differences may occur as a result of the response of dopamine to ovarian hormone fluctuation during the estrous cycle. In rats, the estrus cycle lasts for approximately 4 days, passing through three stages: Diestrus, Proestrous, and Estrus (Butcher & Kirkpatrick-Keller, 1984). It is empirically possible to define this by swabbing vaginal cells and examining these cells microscopically. During the 36 hours of vaginal estrus, the epithelial cells are seen to be cornified, meaning they have accumulated keratin and died. Under a microscope, these cells appear larger and rectangular in shape. Estrus is followed by a period of time in which the number of cornified cells drops, which is termed vaginal diestrus. This stage lasts 2 days, which are termed diestrus stage I and diestrus stage II respectively. After diestrus the number of nucleated cells in the vaginal epithelium increases in comparison to the cornified cells. This stage is known as vaginal proestrus, which is the stage corresponding to behavioral estrus. During this 12-hour period, estrogen levels increase and the animal begins to exhibit estrus behavior.

Studies have shown that, during this period, females rats are much more sensitive to the effects of amphetamine, both chemically and behaviorally. For example, when the dopamine system in the striatum is stimulated during behavioral estrus, female rats exhibit much greater dopamine-associated behaviors, such as rotational behavior, grooming, head-bobbing, and forelimb movements, in comparison to diestrus I (Becker & Cha, 1989; Becker, Robinson, & Lorenz, 1982; Kalivas & Stewart, 1991; Robinson et al., 1980). It is believed that estrogen acts to increase calcium currents in striatal neurons. This, in turn, decreases GABA release bringing about a decreased inhibitory response of dopamine terminals. As a result, dopamine release, metabolism, and reuptake is enhanced indicating greater dopamine activity (Becker, 1999; Robinson et al.).
The enhanced dopamine function in females that is augmented during the estrus cycle prompts locomotor activity to a greater degree than is found in male rats. For example, research has demonstrated that females are more behaviorally sensitive to activation of the D1 receptor, whereas males are more sensitive to activation of the D2 receptor. The effects of these different receptors leads to locomotor activation in the female rat, which contrasts the locomotor depression seen in males (Schindler & Carmona, 2002). Additionally, when male and female rats were injected with a D1 agonist there was a period of locomotor depression followed shortly after by a period of locomotor activation. In this particular study, it was found that males seemed to be much more sensitive to the period of locomotor depression whereas females demonstrated a much greater period of subsequent locomotor activation (Heijtz, Beraki, Scott, Aperia, & Forssberg, 2002). Ultimately, it can been concluded that females are more sensitive to locomotor stimulation brought about via activation of the D2 receptor (Frantz & Van Hartesveldt, 1999; Szumlinski, Goodwill, & Szechtman, 2000). Accordingly, females who receive amphetamine demonstrate increased activity due to the agonizing effects of amphetamine on the dopamine system (Camp & Robinson, 1988; Forgie & Stewart, 1993).

Microdialysis Technique

*In vivo* microdialysis is a useful technique for measuring concentrations of many substrates in the brain of an awake, behaving animal. Recently, microdialysis was used to measure dopamine concentrations of D2 primed animals following acute administration of amphetamine. In this study, D2 primed animals demonstrated a 5-fold increase in striatal dopamine concentration following a single dose of amphetamine (Nowak et al., 2001).

Based on the tenets of sensitization previously addressed, these increases should become even more robust following repeated administrations of amphetamine. This sort of interaction
may convey additional vulnerability to psychostimulant addiction as seen in the schizophrenic population. Additionally, these results were found in the striatum, an area of the brain primarily associated with regulation of motor function that is influenced by other areas more closely associated with learning and reinforcement such as the nucleus accumbens. So, it is possible that the increases in dopamine concentration may be even more robust in brain areas directly related to learning and reinforcement.
CHAPTER 2

METHODS

Collection of Preliminary Data

Treatment groups. Eight research groups were used for this study, each group being comprised of 6-9 male or female Sprague-Dawley rats that were the offspring of timed female dams (Harlan, Indianapolis, IN).

All groups received neonatal injections from postnatal days (P) 1-11 with either saline or the D2/D3 agonist quinpirole. During behavioral sensitization and microdialysis, animals were given ip injections of either saline or d-amphetamine. The experimental design is outlined in Table 1.

Table 1.

Combinations of Sex, Model, and Sensitization Treatments

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Sex</th>
<th>Neo-Natal Treatment</th>
<th>Sensitization Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Male</td>
<td>Quinpirole</td>
<td>Saline</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>Quinpirole</td>
<td>d-Amphetamine</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>Saline</td>
<td>Saline</td>
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<tr>
<td>8</td>
<td>Male</td>
<td>Saline</td>
<td>d-Amphetamine</td>
</tr>
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</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>Saline</td>
<td>d-Amphetamine</td>
</tr>
</tbody>
</table>
**Neonatal injections.** Neonatal injections began on P1, the day following birth, and given each day through P11. All animals received an interperitoneal (i.p.) injection of either 1 mg/kg quinpirole or saline at 1% of the treatment group’s average weight. Following injection, the needle remained in the peritoneal cavity for 3-5 seconds to allow for proper absorption of the drug, avoiding possible capillary action generated by withdrawal of the needle.

At postnatal day 21, animals were weaned, socially housed, and then raised to adulthood (P60). At P60 D2 receptor priming was verified by testing for increased yawning behavior (Cooper, Rusk, & Barber, 1989). Prior to testing, each animal was administered an acute i.p. injection of 100 μg/kg quinpirole and then placed in an inverted cage without bedding as the presence of bedding can induce chewing and nesting behaviors that can supercede yawning. An observer then recorded yawning activity of each rat for a period of 1 hour.

**Behavioral Sensitization.** On the day following the yawning procedure, animals began amphetamine sensitization. During sensitization testing, locomotor activity was tracked every other day for a total of seven exposures to amphetamine or saline. The rationale for using an intermittent sensitization paradigm is based on past studies that have shown repeated intermittent exposure to psychostimulants produces long-term enhancements in the ability of psychostimulants to produce increases in locomotion as compared to saline controls (Robinson & Camp, 1987; Saito et al., 2005; Steketee, 2005).

Before each session animals were weighed and injected i.p. with either saline or d-amphetamine (1.0 mg/kg). Approximately 10 minutes after the injection, animals were placed into one of four identical 15”x15” plywood boxes interiorally painted black to allow for necessary contrast between the white animal and the floor of the apparatus as needed for behavioral tracking with Anymaze Digital Tracking software (Stoelting Inc., Wood Dale, IL).
Before sensitization testing, all animals were given four drug free 10 minute trials to habituate to
the sensitization arena and injection procedures, thus preventing any effects of novelty on
behavioral measures. Approximately 10 min before each habituation trial, all animals were
given an injection of saline, which was done to habituate animals to the injection itself. All
behavioral testing trials were digital video recorded and all movements were analyzed using the
Anymaze software program. Locomotor activity was recorded as the number of line crosses by
each animal, as a grid was superimposed on the digital video file by the software. Other
measures of stereotypic behavior, immobility and rotational spinning behavior, were measured
and compared for directionality of spinning.

Explanation of dependent measures. Horizontal activity is the first dependent measure
under analysis in this study and is the most common dependent measure in studies analyzing
locomotor sensitization. When the animal is placed into the locomotor arena, the AnyMaze
software program digitally superimposes a 5 x 5 cm grid across the floor of the apparatus. Each
time the animal crosses a line of the grid AnyMaze, this even is recorded as an activity count.
The total line crosses for each trial provide a quantification of horizontal activity. The total
number of horizontal activity counts were averaged across subjects within each group on each
day of drug administration. However, this measure can reach a point of diminishing returns. If
dopamine activation is high enough, the animal may enter stereotypy in which behaviors such as
chewing, grooming, and paw treading supercede locomotion. In this case, the animal is recorded
as being immobile. Therefore, measures of stereotypy and immobility will be considered as
well.

Path stereotypy is the second behavioral dependent measure under analysis in this study.
This is another measure of possible dopamine activation. AnyMaze can record 360° rotations
made by each animal. The total number of clockwise and counterclockwise rotations executed by each animal were summed together to compose a variable we referred to as path stereotypy. This is a measure of stereotypic behavior that has also been referred to as checking behavior. Checking behavior is a compulsive stereotypic behavior that is a ritual-like motor activity pattern that has been related to increases in dopamine function, especially increases of dopamine D2 receptor activation (Szechtman et al., 2001; Szechtman & Woody, 2004).

The amount of time spent immobile is the third behavioral dependent measure under analysis. AnyMaze records this event as any time the animal fails to cross a line on the digital grid for a period to 2 seconds. After the 2-second interval, AnyMaze records the time until the next line crossing. This time between the 2-second interval and the next line crossing is time spent in immobility. Although this is essentially the opposite dependent measure as horizontal activation, it does have some unique properties. First, through observation, we have noted that when rats stop moving in the locomotor arena, they typically begin participating in behaviors that have been referred to as stereotypic behaviors such as grooming, paw treading, or vertical rearing. Increases in any of these behaviors has been related to increases in general dopaminergic function (Beninger, Mazurski, & Hoffman, 1991). Second, we have also noted that immobility time and horizontal activity are not necessarily inversely related; in other words, group differences in horizontal activity does not necessarily indicate group differences in immobility time, suggesting these two behavioral measures are independent.

**Verification of estrus cycle.** On each day of testing, stage of estrus cycle of female rats was verified. A sample of cells were taken from the animal’s vaginal lumen using a moistened cotton swab. The sample was then smeared onto a slide and placed under a microscope (Olympus microscope BH series) for analysis. The stage of the estrous cycle was statistically
correlated with the behavioral testing and dopamine release on the amphetamine challenge to
determine if the stage of the estrous cycle interacted with locomotor activity and/or dopamine
release under the influence of amphetamine.

Statistical analysis. Analysis of Variance (ANOVA) was the primary statistical test and
Fisher’s Least Significant Difference test will serve as the post hoc test. Alpha-level was set at
.05 for all analyses. For analysis of behavioral sensitization and dopamine release on the
amphetamine challenge, the design consisted of three independent variables: Sex (Between
subjects, 2 levels: Male, Female), Neonatal Drug Treatment (Between subjects, 2 levels:
Quinpirole and Sal), and Adulthood Drug Treatment (Between subjects, 2 Levels: Sal-Amph,
Sal-Sal). Days 1 and 7 will be analyzed separately to insure a significant change in activity over
days of sensitization training by using a repeated measures ANOVA using days 1 and 7 as the
level of a fourth IV.

Preliminary Data

A 2 x 2 x 7 three-way ANOVA revealed a significant adulthood drug treatment main
effect F(1,35) = 9.35, p<.004, a significant two-way interaction of Adulthood Drug Treatment x
Day of Testing F(6,35) = 4.47, p<.001, and a significant three-way interaction of Ontogenetic
Drug Treatment x Adulthood Drug Treatment x Day of Testing F(6,35) = 3.52, p<.003 on
rotational behavior. Group Q-A demonstrated a significant increase in activity at days 1, 4, and 5
as compared to all other groups (indicated by **). Group S-A demonstrated a significant increase
in activity as compared to controls at days 5-7 (indicated by *). Group S-A also demonstrated a
significant increase in activity from day 1 to day 7, whereas Group Q-A did not, demonstrating
that D2-primed females did not sensitize to amphetamine, whereas controls demonstrated
sensitization to adulthood amphetamine treatment. See Figure 1 for females and Figure 3 for males.

** Q-A animals significantly different from all other groups on days 1, 4, & 5

* S-A animals significantly different from S-S on days 5, 6, & 7

** Figure 1: Activity Counts are Presented as a Function of Drug Treatment Group for Females

These results indicate that although D2-primed females do not demonstrate augmented sensitization compared to nonprimed controls, they do exhibit robust increases in activity on the first day of treatment, which indicates that D2-primed subjects may be more sensitive to the effects of the drug when compared to nonprimed controls. It is important to note, however, that these data have not yet been correlated with and corrected for the estrus cycle data, which may influence our results with further analysis.

A 2 x 2 x 7 repeated measures ANOVA revealed only a significant main effect of adulthood drug treatment F(1,35) = 8.56, p<.006 and a significant main effect of day of testing F(6,35) = 2.81, p<.012, on rotational stereotypy. Amphetamine increased path stereotypy in
females as compared to saline treatment, but there were no effects of ontogenetic drug treatment. Again, it is important to note that the correlation of estrus data could change the significance of this outcome. Additionally, as there are only 3-4 animals per treatment group, continued testing may help lower variability, increase statistical power, and bring out the apparent differences in the means. See Figure 2.

Figure 2: Total Number of Clockwise and Counterclockwise Turns as a Function of Drug Treatment for Females.
** Q-A animals significantly different from all other groups on days 1-6 of testing

* S-A animals significant from saline controls on days 3-7, Q-A also significantly different from only control on day 7

** Figure 3: Activity Counts are Presented as a Function of Drug Treatment Condition for Males.**

A 2 x 2 x 7 repeated measures ANOVA revealed a significant main effect of adulthood drug treatment F(1,31) = 28.62, p<.001 and a significant main effect of day of testing F(6,31) = 3.31, p<.004 and significant two-way interactions of Ontogenetic Drug Treatment x Adulthood Drug Treatment F(1,31)= 6.71, p<.015, and Adulthood Drug Treatment x Day of Testing F(6,31) = 5.14, p<.001 on male rotational behavior. Adulthood amphetamine treatment produced a significant increase in the path stereotypy of D2-primed males on days 1-5 and day 7, and amphetamine increased path stereotypy on days 4-7 in non D2-primed males but only to the levels of D2-primed subjects. See Figure 4.
** Q-A significantly different from all other groups on days 1-4, 7

* S-A significantly different from control on days 4-7, Q-A significantly different from control on days 4-5

Figure 4: Number of Clockwise and Counterclockwise Turns as a Function of Drug Treatment for Males.

Discussion of Preliminary Results and Modification of Final Methods

Preliminary results indicate that, for both sexes, D2 primed subjects receiving amphetamine demonstrate significant increases in locomotor activity when compared to non-D2-primed amphetamine controls. Earlier literature suggests that dopamine dysregulation leads to the increases of extracellular dopamine that underlie such robust increases in locomotor activity (Kalivas & Duffy, 1993; Kalivas et al., 1998; Kalivas et al., 1993; Nowak et al., 2001). Interestingly, however, males demonstrate higher activity overall. This may indicate that either males are more sensitive to the behavioral effects of amphetamine, contradicting a substantial amount of earlier literature (Becker, Molenda, & Hummer, 2001; Booze et al., 1999) or females
are differentially affected by amphetamine to the extent that they reach a high level of stereotypy (Melnick & Dow-Edwards, 2001) that may be superceding horizontal activity.

The results of the path stereotypy data seem to implicate the former of these possible outcomes, as D2 primed males demonstrate a significantly higher number of rotations compared to nonprimed controls while females are not statistically increased on the same measure. This result, again, contradicts previous literature that suggests females are more prone to demonstrate stereotypic behavior (Melnick & Dow-Edwards, 2001). However, it is important to note that procedures by Melnick used cocaine, not amphetamine. So, it is possible that amphetamine is inducing such a high dopaminergic response in female subjects that the behavioral response is reaching a point of diminishing returns. That is, female subjects are spending much more time immobile while performing other superceding behaviors such as head bobbing, chewing, paw treading, and licking, that are not able to be tracked using digital methods. Microdialysis procedures will be used to measure changes in dopamine release.

*Experimental hypotheses.* Based on the rationale above, I hypothesize that D2 primed subjects show higher extracellular levels of dopamine as compared to nonprimed controls also receiving amphetamine. Additionally, I hypothesize that females will demonstrate increased levels of extracellular dopamine in comparison to male subjects as previous literature would suggest. In order to verify the validity of our female behavioral results, data for time spent in immobility as well as total number of immobile episodes during the sensitization procedure were gathered and analyzed. I hypothesize that, in addition to D2 primed animals spending less time in immobility, females will spend more time, overall, in immobility as compared to male subjects, which would help to explain the lower level of horizontal and rotational activity seen for females in the preliminary data. See Tables 2 and 3 for treatment groups.
Surgical implantation of the guide canula. On the day following the final amphetamine or saline sensitization trial, each animal was anesthetized using a 60% to 40% solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). Once the animal was anesthetized, the head was shaved and the animal was placed into a stereotaxic frame (Kopf, Instruments, Tujunga, CA). Once in the frame, the animal’s nose was placed into a Kopf stereotaxic mask (Model 906, Kopf Instruments, Inc., Tujunga, CA) that was attached to a gas anesthesia machine (JD Medical VT-100, Phoenix, AZ), which constantly delivered an isoflurane/O2 gas mixture to the animal to help maintain anesthesia. A single midline scalp incision was made to expose the skull, which was cleaned with an alcohol wipe and dried to allow for maximal adherence of the acrylic cap. Once the skull was clean, a surgical drill was mounted on the stereotaxic frame and used to penetrate the skull. The drill was moved to the coordinates AP (anterior/posterior) = + 1.6 mm, ML (medial/lateral) = +/- 1.2 mm. Three other shallow indentions were drilled around the skull penetration, and surgical screws were cemented into these indentions to serve to anchor the acrylic cap onto the skull. At this point, the drill was removed and a guide canula was attached by a holder to the stereotaxic frame. The canula was then lowered through the hole to the surface of the exposed dura matter. The canula was then lowered ventrally 6.0 mm so that it would be located directly above the nucleus accumbens core. Liquid acrylic was then placed onto the skull around the canula and anchor screws. The acrylic was placed so that it covered the screws and reached the structural plastic portion of the canula. Once the acrylic dried, the incision was closed using sutures and surgical staples.

During the postoperative period, the animal was removed from the stereotaxic frame and placed onto heating pads in a bedding free cage. A 10 cc subcutaneous (s.c.) of saline was administered along with an intramuscular (i.m.) injection of 1 mg/kg ketoprophen anti-
inflammatory to help with pain management. When the animals were awake and ambulatory, animals were returned to their home cage and allowed to recover for 6 days during which time they were weighed and monitored daily.

*Microdialysis amphetamine challenge.* Seven to 10 days following surgery, the animal was weighed and the estrous cycle was recorded in female rats. The stylus was then removed from the guide canula and the probe inserted through the guide cannula. Once the probe was secured onto the guide cannula, a filtered solution of artificial cerebrospinal fluid was dialyzed through the probe at a rate of 2.0 μl/minute. Samples were taken every 20 minutes and placed into a conical centrifuge vial along with 1 ml of 0.5 M perchloric acid that will serve to denature proteins that can break down the neurotransmitters targeted in this procedure. At this point, the samples were placed into a -80°C freezer until which time they can be analyzed using High Performance Liquid Chromatography (HPLC).

Samples were collected in this manner for the first 2 hours of the procedure. At the beginning of the 3rd hour, animals began a series of four injections of saline or d-amphetamine according to the treatment received during sensitization, which was given at 20-minute intervals. Animals receiving injections of amphetamine were dosed cumulatively starting at 0.1 mg/kg increasing to 0.6 mg/kg, 1.1 mg/kg, and 2.6 mg/kg over the respective 20-minute intervals. Samples were collected every 20 minutes over the final 3 hours of the procedure.

*HPLC analysis of samples.* Before experimental samples were analyzed, a series of standards of dopamine (DA), norepinepherine (NE), DOPAC, and homovanillic acid (HVA) were prepared at various known concentrations to construct a standard curve from which concentrations of the target neurotransmitters were calculated using the equation of the curve. Additionally, pure standards were injected onto the column to determine the retention time of
each neurotransmitter. The samples being tested were allowed to defrost to liquid phase and were then pipetted into vials to be placed into an autosampler (ESA, Chelmsford, MA). The autosampler then injected 40 µl of each sample onto the detection column (ESA, Chelmsford, MA), where the sample separated into its respective constituents that cleared the column at its respective retention time. This data are represented in a spectrograph for each sample analyzed. The area of each target peak was compared to the standard curve to calculate concentrations of each target neurotransmitter.

Statistical analysis. Dopamine release, as measured by HPLC was analyzed as the percent change above baseline for of each sample. Locomotor activity was analyzed as line crossing activity across days. A 2x2x2x7 (sex x D2 priming x adult drug treatment x day) ANOVA with a Newman-Keuls post hoc test was run to determine main effects, if any, on horizontal activity, path stereotypy, and immobility.
Table 2.

*Final Numbers of Subjects in Each Treatment Condition for Behavioral Measures*

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Sex</th>
<th>Neo-Natal Treatment</th>
<th>Sensitization Treatment</th>
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<tbody>
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<td>9</td>
<td>Male</td>
<td>Quinpirole</td>
<td>Saline</td>
</tr>
<tr>
<td>9</td>
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<td>Quinpirole</td>
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<tr>
<td>8</td>
<td>Female</td>
<td>Saline</td>
<td>d-Amphetamine</td>
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</tbody>
</table>

Table 3.

*Final Numbers of Subjects in Each Treatment Condition for Microdialysis ProcedureCollapsed Across Sex*

<table>
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<th>Number of Animals</th>
<th>Neo-Natal Treatment</th>
<th>Sensitization Treatment</th>
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<tbody>
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<td>10</td>
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<tr>
<td>9</td>
<td>Saline</td>
<td>d-Amphetamine</td>
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</table>
Amphetamine Sensitization

*Overall horizontal activity.* In the initial analysis of each of the dependent measure, all independent variables were included: sex, ontogenetic drug treatment, adulthood drug treatment, and day of testing. If sex differences were revealed, we analyzed males and females separately. When no sex differences were revealed, we collapsed over the independent variable. A 2 (sex) x 2 (neonatal drug treatment) x 2 (adulthood drug treatment) x 7 (day of testing) four-way ANOVA revealed significant main effects for adult drug treatment, \( F(1,71) = 2.89, p<.001 \), and day of testing, \( F(6,71) = 2.44, p<.025 \), three significant two-way interactions, including neonatal drug treatment x adulthood drug treatment, \( F(1,71) = 7.73, p<.007 \), sex x day of testing, \( F(6,71) = 2.38, p<.029 \), and adulthood drug treatment x day of testing, \( F(6,71) = 11.21, p<.001 \), and a three-way interaction of sex x neonatal drug treatment x adulthood drug treatment \( F(6,71) = 3.32, p<.003 \). Analysis of the main effects showed that d-amphetamine produced a significant increase in horizontal activity as compared to saline-treated controls, and there was a significant increase of horizontal activity over the 7 days of testing. Post hoc analysis of the neonatal drug treatment x adulthood drug treatment interaction revealed that D2-primed animals given amphetamine demonstrated a significant increase in horizontal activity compared to controls. Post hoc analysis of the sex x day of testing revealed that males demonstrated a significant increase in horizontal activity relative to females but only on day 6. Finally, post hoc analysis of the significant adulthood drug treatment x day testing interaction revealed that d-amphetamine produced a significant increase in activity from days 2-7. Most importantly, analysis of the three-way interaction revealed that D2-primed males demonstrated a significant increased
response to amphetamine as compared to all other groups. This result shows that D2-priming as produced by ontogenetic quinpirole treatment enhanced locomotor activation to amphetamine compared to nonprimed controls. Horizontal activity is presented for females in Figure 5a and males in Figure 5b.
(a) Females

(b) Males

** Indicates significantly greater than all other groups

* Indicates significantly greater than controls

Figure 5: Activity Counts for Females 5(a) and Males 5(b) as a Function of Drug Treatment Group and Day of Testing. Group Q-A exhibited significantly higher levels of horizontal activity than all other groups on days 1, 4, & 5 Group S-A demonstrated significantly higher levels of horizontal activity as compared to controls on days 2-7. (b) Activity counts for males
as a function of drug treatment and day of testing. Group Q-A exhibited significantly higher horizontal activity levels as compared to all other groups on days 1-7 of sensitization testing; Group S-A demonstrated significantly higher levels of horizontal activity as compared to Groups S-S and Q-S on days 3 through 7.

_Horizontal activity, males._ Based on the significant interactions involving sex as a factor, males and females were analyzed separately. For males, a 2 x 2 x 7 three-way ANOVA on horizontal activity revealed significant main effects of adulthood drug treatment, \( F(1,37) = 31.17, p<.001 \), and day of testing, \( F(6,37) = 4.78, p<.001 \), as well as significant two-way interactions for neonatal drug treatment x adulthood drug treatment, \( F(1,37) = 6.65, p<.014 \), and adulthood drug treatment x day of testing, \( F(6,37) = 6.11, p<.001 \). Analysis of the significant main effects revealed that amphetamine-treated animals increased horizontal activity compared to saline-treated controls and there was a significant increase in activity over days of testing. Analysis of the significant two-way interaction of adulthood drug treatment x day of testing revealed that amphetamine significantly increased activity over time. Most importantly, post hoc analysis of the significant ontogenetic drug treatment x adulthood drug treatment interaction revealed that D2 primed males administered amphetamine demonstrated a significant increase in horizontal activity relative to all other groups, showing that D2-priming as produced by ontogenetic quinpirole treatment enhanced locomotor activation to amphetamine in males.

_Horizontal activity, females._ A 2 x 2 x 7 three-way ANOVA on the horizontal activity in females revealed a significant main effect for adulthood drug treatment, \( F(1,39) = 14.38, p<.001 \), a significant two-way interaction of adult drug treatment x day of testing, \( F(6,39) = 6.0, p<.001 \), and a significant three-way interaction of neonatal drug treatment x adulthood drug treatment x day of testing, \( F(6,39) = 2.90, p<.001 \). Amphetamine increased horizontal activity
compared to controls Post hoc analysis of the adulthood drug treatment x day of testing revealed that amphetamine increased activity compared to controls throughout testing. Analysis of the significant three-way interaction revealed that D2 primed females administered amphetamine demonstrate a significant increase in horizontal activity on days 1, 4, and 5 compared to all other groups. This result indicates different patterns of locomotor activation in response to amphetamine in males and females, with males demonstrating more overall activation on the horizontal activity measure than females.

Path stereotypy. Path stereotypy was collapsed across sex and day of testing as no differences were found on these variables. D2 primed animals given amphetamine exhibit significantly higher path stereotypy than all other groups. See Figure 6.

** indicates significantly greater than all other groups (p<.05)

* indicates significantly greater than controls (p<.05)

Figure 6: Path Stereotypy as a Function of Drug Treatment Group. Group Q-A exhibited higher overall path stereotypy than all other groups; Group S-A exhibited significantly higher levels of path stereotypy as compared to Groups Q-S and S-S.
Overall immobility time. Immobility time is presented as a function of drug treatment group in Figures 7(a) for females and 7(b) for males. A 2x2x2x7 ANOVA including all four factors on immobility time revealed a significant main effect of adulthood drug treatment, $F(1,72) = 63.81$, $p<.001$, significant two-way interactions for neonatal drug treatment x adulthood drug treatment, $F(1,72) = 8.1$, $p<.006$, adulthood x day of testing, $F(6,71) = 3.39$, $p<.003$, and sex x day of testing, $F(6,71) = 4.61$, $p<.001$, as well as a significant three-way interaction for sex x neonatal drug treatment x day of testing, $F(6,71) = 2.95$, $p<.008$. Saline-treated animals demonstrated a significant overall increase in immobility time, as was expected. Post hoc analyses of the significant two-way interactions revealed that D2-primed animals administered amphetamine were less immobile than controls, amphetamine-treated animals spent less time immobile than controls on days 2 through 7, whereas males spent more time immobile than females on days 1 and 2 of drug treatment. Most importantly, post hoc analysis of the significant three-way interaction revealed that D2-primed males given amphetamine spent less time immobile than all other groups. Supporting the analysis of the horizontal activity data, D2-primed males demonstrated more locomotor activation than all other groups.

Immobility time, males. A 2 x 2 x 7 three-way ANOVA on male immobility time revealed a significant main effect of neonatal drug treatment, $F(1,36) = 7.33$, $p<.01$, adulthood drug treatment, $F(1,36) = 55.43$, $p<.001$, and day of testing, $F(6,36) = 3.46$, $p<.003$, and significant 2-way interactions of neonatal drug treatment x adulthood drug treatment, $F(1,36) = 10.88$, $p<.002$, and adulthood drug treatment x day of testing, $F(6,36) = 4.03$, $p<.001$. Finally, this analysis also revealed a significant 3-way interaction of neonatal drug treatment x adulthood drug treatment x day of testing $F(6,36) = 2.83$, $p<.01$. Overall, D2-primed males and amphetamine-treated males spent less time immobile than male rats neonatally treated with
saline or treated with saline in adulthood respectively. Post hoc analyses of the significant two-way interactions revealed that D2-primed males given amphetamine spent less time immobile than all other groups, adulthood amphetamine treatment spent less time immobile than saline-treated controls from days 2 through 7. Finally, and most importantly, D2-primed males administered amphetamine spent more time immobile than all other groups on days 1 through 6. These data reinforce the locomotor activation data that D2 primed males administered amphetamine demonstrate a significant increase in activity compared to all other groups throughout most of drug treatment, much unlike D2-primed females that demonstrated a different pattern of activation due to drug treatment. See Figure 7b.

*Immobility time, females.* Based on the fact there were several statistically significant sex effects, males and females were analyzed separately on immobility time. A 2 x 2 x 7 three-way ANOVA on female immobility time revealed significant main effects of adulthood drug treatment, \(F(1.35) = 22.10, p<.001\) and day of testing, \(F(6,35) = 2.17, p<.04\). Overall, amphetamine-treated females spent less time immobile than saline-treated females, and there was a change in immobility time over days of testing. Although there were no significant interactions in females, it did appear through observation that there may be significant group differences on the first day of drug treatment. Therefore, we ran an additional 2x2 ANOVA on day 1 of drug treatment with only neonatal drug treatment and adult drug treatment as factors and found a significant two way interaction of these variables, \(F(1,36) = 4.91, p<.033\). Thus, it was determined that D2 primed females receiving amphetamine spent less time immobile than all other groups on the first day of adulthood drug treatment, suggesting an initial increase in activity in D2-primed females produced by amphetamine. See Figure 7a.
**Figure 7:** Time Spent in Immobility for Females 7(a) and Males 7(b) as a Function of Drug Treatment Group and Day of Testing. For females, group Q-A exhibited significantly decreased immobility time compared to all other groups on Day 1, and Groups Q-A and S-A exhibited significantly greater immobility time compared to all other groups.
significantly less time immobile as compared to Groups Q-S and S-S on days 2 through 7. 7(b):
For male, Group Q-A exhibited decreased immobility as compared to all other groups on days 1-3 and 5-7, and Group S-A demonstrated significantly less time immobile on days 3 through 7.

Dopamine High Performance Liquid Chromatography (HPLC) of Cerebrospinal Fluid Microdialysate

Dopamine release in the nucleus accumbens core is presented as a function of drug treatment condition in Figure 8. No statistically significant sex differences in dopamine release were present in these data, so data were collapsed over that factor. A 2 x 2 x 9 three-way ANOVA revealed significant main effects for neonatal drug treatment, F(1,40) = 5.93, p<.019, and adulthood drug treatment, F(1,40) = 13.03, p<.001, as well as significant two-way interactions for neonatal drug treatment x adult drug treatment, F(1,40) = 5.63, p<.022 and adulthood drug treatment x time, F(8,40) = 2.68, p.004. Both neonatal quinpirole treatment and adulthood amphetamine treatment significantly increased dopamine release from time point 1 through 8, as expected. More importantly, post hoc analyses revealed that D2-primed rats administered amphetamine demonstrated a significant increase in dopamine release compared to all other groups. See Figure 8.

Due to the high variability among D2 primed females receiving amphetamine, no sex effects were observed. However, through observation, we noticed that females demonstrated an absolute increase of dopamine release, and this increase was approximately 41% in D2-primed females administered amphetamine as compared to non D2-primed males given amphetamine, whereas non D2-primed females administered amphetamine demonstrated a smaller 5% increase in dopamine release after amphetamine treatment a compared to non D2-primed males. Although
this was not a statistically significant effect, it suggests that a sex difference may exist, and currently, more subjects are being added to these groups to increase statistical power.

** indicates significantly greater than all other groups

* indicates significantly greater than controls

Figure 8: Percent Increase From Baseline Levels of Dopamine Release in the Nucleus Accumbens as a Function of D2 Priming and Adult Drug Treatment as Quantified by High Performance Liquid Chromatography. Group Q-A demonstrate a significant three-fold increase in accumbal dopamine release as compared to Group S-A. Group S-A demonstrated an approximate 400% increase in accumbal dopamine release as compared to baseline.
CHAPTER 4

DISCUSSION

Interaction of Neonatal Treatment With Quinpirole and Adulthood Drug Treatment With d-
Amphetamine

The results from this study showed that rats neonatally treated with the dopamine D2/D3 agonist quinpirole demonstrated enhanced locomotor activation to d-amphetamine as adults. Additionally, priming of dopamine D2 receptors enhanced the dopaminergic response in the nucleus accumbens core to d-amphetamine when animals were given an amphetamine challenge 7 days after sensitization testing was complete. Therefore, it appears that priming of the dopamine D2 receptor leads to an enhanced dopaminergic response to the psychostimulant amphetamine not witnessed in nonprimed animals in a brain area that is known to mediate the behavioral activating effects of drugs with hedonic properties. These findings suggest that this enhanced dopaminergic response would lead to an increased behavioral activation in a D2-primed system and suggesting that priming of dopamine D2 receptors could lead to a propensity to use psychostimulants such as amphetamine.

Behavioral measures, horizontal activity. It was hypothesized that animals neonatally treated with quinpirole would demonstrate an augmented behavioral response in terms of horizontal activity. Results supported this hypothesis in that both male and female D2-primed animals that received amphetamine during sensitization demonstrated a significantly greater number of line crosses compared to all other treatment groups beginning on the 1st day of drug exposure. The difference between these groups later dissipates as nonprimed animals sensitize to amphetamine. Past research has shown that modifications in dopamine function are critical for sensitization to several psychostimulants, including amphetamine, nicotine, and cocaine (Kalivas
Specifically, increased dopaminergic function in the mesocorticolimbic pathway has been hypothesized to play a significant role in behavioral sensitization (Kalivas et al.). These results support the claim that D2 priming does indeed lead to a supersensitized response of dopaminergic neurons to a stimulant drug, as shown in Nowak et al. (2001), and this increase in dopaminergic function appears to be manifested in a significant increase in horizontal activity.

Path stereotypy. It was hypothesized that D2 primed animals receiving d-amphetamine during sensitization would demonstrate a higher number of clockwise and counterclockwise rotations after amphetamine treatment. The finding that D2 primed animals administered amphetamine exhibit significantly greater overall rotational behavior when compared to all other groups. A significant increase in stereotypic behaviors again suggests a hyperactive dopaminergic response to amphetamine and support the horizontal activity data in showing that there appears to be a significantly increased behavioral activation to amphetamine in D2-primed animals. (Kostrzewa, Kostrzewa, Nowak, Kostrzewa, & Brus, 2004). The analysis of this particular stereotypic behavior is unique, as most studies have shown an increase in stereotypic behaviors after dopaminergic agonists, which typically includes grooming, paw treading, and vacuous chewing movements. Path stereotypy is a behavior that has also been referred to as compulsive checking (Szechtmann et al., 2001), and the increase in this behavior has also been associated with increased dopaminergic activity and obsessive-compulsive disorder.

Immobility time. It was hypothesized that D2 primed animals would spend less time in immobility in response to d-amphetamine than would nonprimed animals. These data demonstrate that D2 primed animals exhibit significantly less time in immobility in response to
amphetamine when compared to all other treatment groups. This also supports the claim of increased dopaminergic activation in these animals.

**Behavioral augmentation as a result of D2 priming.** Studies have shown that dopamine release in the nucleus accumbens is necessary for the behavioral reinforcement and subsequent induction or initiation of behavior in response to a drug (Cador et al., 1995; Hooks et al., 1992). More specifically, blockade of the either the dopamine D1 (Vezina & Stewart, 1989) or D2 receptor has been shown to attenuate induction of behavioral sensitization, and it has been hypothesized that the D2 receptor is necessary for such behavioral expression (Chiang et al., 2003; Feldpausch et al., 1998). If D2 primed animals exhibit an augmented behavioral response when exposed to d-amphetamine, one could realistically conclude that levels of dopamine in the nucleus accumbens will be substantially elevated as a result of the increased activation of primed D2 receptors in the nucleus accumbens. Certainly, the data from microdialysis support this conclusion. These data are consistent with studies in both animals (Kalivas & Duffy, 1993; Kalivas et al., 1993; Vanderschuren & Kalivas, 2000) as well as what has been shown in human schizophrenics (Abi-Dargham et al., 1998; Laruelle et al., 1996). We assume that the reinforcing action of the dopamine in the nucleus accumbens likely becomes much greater in these individuals and this is the mechanism underlying the increased abuse of psychostimulants in this population.

**Augmented Dopamine Release in the Nucleus Accumbens of D2 Primed Animals Given Amphetamine in Adulthood**

It is thought that the key to D2 receptor supersensitivity has to do with the mechanism of action at the D2 receptor. The D2 receptor is what is known as a metabotropic receptor which is in direct contrast to ionotrophic, or fast synaptic receptors that can affect the state of a neuron.
almost instantly. The time of action for an ionotropic receptor is, for the most part, negligible regarding its effect on the cell. On the other hand, because metabotropic receptors respond to stimuli much more slowly, the time course of action is longer, but changes at these receptors often have a much greater effect on overall neuronal functioning.

This time course of action at metabotropic receptors is dependent upon the execution of a chemical reaction that occurs once the substrate binds the receptor. In a metabotropic receptor, the receptor couples to a protein called the G-protein when it is bound by a substrate. This protein sets off a chain of chemical reactions that, upon their completion, lead to the opening of the transmembrane receptor, which contributes to possible depolarization of the cell. In each step of this chain reaction, certain catalysts are required to carry out the reaction while the presence of other proteins will regulate the reaction. Manipulation of any of these factors could lead to a shift in the time-course of the g-protein coupled receptor.

A recent study published from our laboratory has shown that one of the regulatory proteins that moderates this chain reaction, RGS9, is significantly down regulated in D2 primed animals (Brown, Flanigan, et al., 2004; Maple, Perna, Parlaman, Stanwood, & Brown, 2007). RGS9 is a regulatory protein that has been shown to accelerate the termination of D2 related events (Kovoor et al., 2005). There is also evidence suggesting that cAMP, a catalyst in the second messenger cascade, is upregulated in the sensitized D2 receptor (Self & Nestler, 1998). When accelerated and unmediated, this chain reaction can happen very quickly and with increased frequency. As a result, it can be assumed that the firing frequency of dopaminergic neurons increases as a result of D2 receptor supersensitivity (Kostrzewa et al., 2004), which presumably would be further accelerated when amphetamine, a potent dopamine agonist, is acting at the synapse and increase D2 receptor activity.
In the present study, D2 primed animals exhibited a three-fold increase in accumbal dopamine release over nonprimed animals when both are administered amphetamine. This increase in dopaminergic activity was presumably manifested in behavioral activation produced by amphetamine in D2-primed animals. The behavioral effects of amphetamine are thought to arise due to the drug's ability to increase concentrations of synaptic dopamine. The reasons for this effect are four-fold. First, amphetamine stimulates direct dopamine release from the nerve terminal by upregulating the dopamine transporter that is responsible for exporting dopamine from the cell (Izenwasser & Cox, 1990). Second, amphetamine produces a subsensitization of the D2 autoreceptor, meaning the autoreceptor, which is responsible for attenuating dopamine release at the nerve terminal when synaptic concentrations reach high levels, becomes much less responsive (Henry, Greene, & White, 1989). Third, amphetamine blocks reuptake of dopamine while it also, fourth, inhibits the action of monoamine-oxidase, the enzyme responsible for breaking down dopamine in the synapse (Butcher, Fairbrother, Kelly, & Arbuthnott, 1990). When this effect is combined with a supersensitive response from D2 receptors, then the overall dopaminergic response is increased (Nowak et al., 2001). Nowak et al. showed a robust increase of dopamine in the striatum in D2-primed rats after acute amphetamine treatment, and congruent with this finding, the present results report a robust increase of dopamine in nucleus accumbens core, a brain area located directly ventral to the striatum.

Additionally, to help verify this hypothesis, future studies are aimed to blocking both the D1 and D2 receptors to analyze their specific roles in both locomotor sensitization and dopamine release in D2-primed rats. Past studies have shown that the D1 receptor seems to play more of a role in inducing the behavioral effects of sensitization (Vezina, 1996), whereas the D2 receptor seems to be more involved in the behavioral expression resulting from the induction of
locomotor sensitization incurred from the D1 receptor (Chiang et al., 2003; Feldpausch et al.,
1998). Changes in the sensitivity of the D1 receptor may bring about deregulation of dopamine
release presynaptically (Kalivas & Stewart, 1991) while the effect of D2 receptor
supersensitivity seems to effect dopamine transmission cell both pre- and post synaptically
(Feldpausch et al., ; Nowak et al., 2001). Thus, if the D2 receptors are supersensitized with
neonatal quinpirole robust behavioral augmentation and dopamine release occurs. This may
suggest that the firing rate of dopamine neurons in this area may be further accelerated
following chronic administration of amphetamine than it would be under normal circumstances,
and future studies could analyze this through electrical recording in this region.

One concern brought to light in these data has to do with reconciling these results with
those of Nowak et al. (2001). In this past work, Nowak et al. demonstrated a 4-fold increase in
dopamine release in D2-primed rats in response to an acute dose of d-amphetamine; however,
this change in dopamine release was shown in the dorsal striatum. The dorsal striatum is a
structure directly dorsal to the nucleus accumbens and plays a primary role in skilled motor
movements but also communicates with the nucleus accumbens and has been shown to be
involved in mediating some of the reinforcing actions of drugs (Belin & Everitt, 2008). In the
current study, it was hypothesized that because microdialysis was performed in the nucleus
accumbens, a primary brain structure concerned with positive reinforcement, we would expect a
much larger differential increase in dopamine in D2-primed as compared to non D2-primed rats.
There have been several studies approaching this issue, and the results have been contradictory.
Whereas (Kuczenski & Segal, 1992; Robinson & Camp, 1990) have reported no significant
differences in dopamine release in response to amphetamine in the dorsal striatum as compared
to the nucleus accumbens, DiChiara and colleagues (1993, 2004) have found that amphetamine
induced a larger increase in dopamine release in the core of the nucleus accumbens as compared to the dorsal striatum (Di Chiara et al., 2004). One of the underlying issues that has been suggested has to do with the placement of the microdialysis probe, and it appears that there are more robust increases in dopamine release in the lateral as compared to the medial striatum, and the lateral striatum has more anatomical similarity to the nucleus accumbens (Di Chiara, Acquas, Tanda, & Cadoni, 1993). Regardless, this is an important issue, as the nucleus accumbens has long been hypothesized to be the brain region central to the hedonic effects of drugs.

A second issue with reconciling data with Nowak et al. (2001) was that this past study reported a 5-fold increase of dopamine release in the striatum, whereas the highest increase in dopamine release in the current study was a 3-fold increase in the nucleus accumbens. However, it is important to consider the overall percent increase above baseline in conjunction with the difference between treatment groups. In the experiment by Nowak et al., a 1050% increase in dopamine was observed in the striatum 40 minutes after a 1.0 mg/kg acute dose of d-amphetamine. In this experiment, 40 minutes following a cumulative 0.6 mg/kg dose of amphetamine, we observed a 1300% increase of dopamine release in D2 primed animals. Also, non-D2 primed animals in this experiment demonstrated a 450% increase in dopamine release as compared to the 250% increase observed by Nowak. So, even at a lower dose of amphetamine, animals in the present study demonstrated a significantly higher overall level of dopamine release. Additionally, we notice that dopamine levels actually even before the cumulative dose of amphetamine reaches 2.5 mg/kg, the highest dose employed in this study. It is possible that, with decreased variability, dopamine levels may have even significantly decreased before the highest dose was administered. This could be indicative of a ceiling effect of dopamine release, as dopamine stores may have been depleted, which may help to explain why a higher differential
release was not seen as expected. It must also be noted that these increased levels of dopamine are observed 7 days following withdrawal of the drug. It is expected that this increased dopamine would lead to increased euphoria and, possibly, greater reinforcement if relapse occurs. Attempting to block such a substantial rebound in dopamine levels may be a possible future target for relapse prevention.

*Sex Differences in Amphetamine Sensitization and Accumbal Dopamine Release*

*Behavioral measures horizontal activity.* The hypothesis that females would exhibit greater horizontal activity than males in response to d-amphetamine treatment was not supported in the present study. Actually, D2 primed males demonstrated a significant increased horizontal activity response to amphetamine than all other groups. Additionally, males show a significant overall increase in horizontal activity relative to females on day 6. This finding suggests that dopamine activation among males in this study may actually be higher than that of females, or that females are demonstrating a significant increase in stereotypic behavior that would actually decrease their horizontal activity. Previous studies have reported that females rats are much more sensitive to the effects of amphetamine, both chemically and behaviorally (Becker & Cha, 1989; Becker et al., 1982; Kalivas & Stewart, 1991; Robinson et al., 1980). One possible explanation may be that males have been reported to be more sensitive to behavioral activation of the D2 receptor (Schindler & Carmona, 2001), which could explain the significant increase in activity for D2 primed males.

However, the only time point at which males increase in horizontal activity compared to females is on day 6 of testing. Taking a look at the overall patterns witnessed in these animals, it appears that activity in amphetamine-treated females appears to be fluctuating while activity in amphetamine treated males shows a more continuous increased locomotor response to
amphetamine, which is a more typical pattern of locomotor sensitization to psychostimulants. Horizontal activity in females appears to asymptote on day 5 and decrease on day 6. However, amphetamine-treated males maintain their highest levels of activity on day 6. Previous research suggests that dopamine levels may decrease, as dopamine stores become exhausted following prolonged stimulant exposure (Koob et al., 2004). So, it may be that case that if females are indeed more reactive to the effects of psychostimulants, they may be experiencing this decrease sooner that the males, who do not exhibit any signs of a decrease. This may explain the inverse of the expected seen effect on this day. Alternatively, females may be demonstrating a significant increase in stereotypic behaviors such as grooming, paw treading, and vacuous chewing movements, which would actually decrease horizontal activity but may be a manifestation of increased dopmainergic activity.

Path stereotypy. No significant sex differences were observed on the path stereotypy measure. This finding is contrary to previous research that has suggested females demonstrate increased stereotypy in response to amphetamine; however, it depends on the behaviors that are measured (Becker et al., 2001). On the other hand, there is research to suggest that Sprague-Dawley rats may be much more responsive to stimulants in terms of both horizontal activity and stereotypy (Horowitz, Kristal, & Torres, 1997). If it is the case that our animals are reaching an apex in behavioral responding to amphetamine, the use of Sprague-Dawley rats may be further contributing to obscuring of sex differences in this instance. Further study might seek to investigate these measures in other species where behavioral responding may not be as robust so to allow identification of a differential in responding resulting from sex.

Immobility time. The hypothesis that females would spend a significant increase in time in immobility than males was not supported. It was hypothesized that if females were exhibiting
higher levels of stereotypy, it would result in greater levels of immobility because behaviors such as chewing, grooming, and paw treading are not quantifiable using AnyMaze. However, while these behaviors are occurring, the animals are not recording activity counts as previously discussed, so the animal is considered to be immobile. Males administered amphetamine actually exhibited greater time in immobility compared to females on days 1 and 2, which suggests a higher initial locomotor activation to amphetamine in females. This result supports the claim that females may be more activated by amphetamine after acute administration. Although this has not been shown in past work, it suggests that females may be more behaviorally activated by initial amphetamine treatment.

Dopamine microdialysis. Although D2 primed females exhibited a 41% increase in dopamine release compared to D2 primed males when both are administered amphetamine, no significant sex differences were observed in these data. This is likely due to the high variability observed in the D2 primed females. It is important to note, however, that this variability is not observed in the other treatment groups. We hypothesize that one reason this may be the case is that the level of priming of the D2 receptor may be different across subjects. This observation is supported in the literature through the yawning test. Past studies from our lab have reported some variability in yawning of D2-primed subjects, ranging from as low as 8-22 yawns in an hour in males, and approximately 3-7 yawns in an hour in females (Brown et al., 2002, 2004; Maple et al., 2007). These result suggests some variability in the sensitivity in the dopamine D2 receptor across subjects and across studies (Cooper et al., 1989; Kostrzewa et al., 1995). This being the case, it is uncertain whether increasing the number of subjects will enhance statistical power sufficiently to observe a significant sex difference in these data. Nonetheless, this supports the claim that there is extensive dysfunction in D2 primed females administered amphetamine,
which may help to explain why schizophrenic females seem to be differentially affected by psychostimulants in comparison to schizophrenic males (Gearon & Bellack, 2000). It is also suggests that our model may represent both mild and severe forms of schizophrenia, at least based on sensitivity of the D2 receptor.

**Conclusion**

It appears neonatal treatment with quinpirole resulting in supersensitivity of the D2 receptor produced increased behavioral and neurochemical measures when animals were chronically exposed to amphetamine. This suggests these animals may be differentially vulnerable to psychostimulant addiction. Additionally, assuming D2 supersensitivity is a potential model of the dopamine dysfunction witnessed in schizophrenia, this interaction of D2 neonatal drug treatment increasing the effects of adulthood drug treatment may shed light on the schizophrenics’ propensity to abuse psychostimulant drugs.

It is uncertain if sex differences play as prominent of a role in this interaction. Females may indeed be differentially responsive but further experimentation is required. It appears that females may be more acutely affected by amphetamine than males as evidenced by decreases in immobility on the initial day of treatment. Additionally, horizontal activity in females appears to reach peak levels more quickly than is seen in males. This would suggest that dopamine release in females may be more robust initially than in males. However, as a result, dopamine may become more quickly depleted in females. Nonetheless, the high levels of variability in female D2 primed animals given amphetamine indicates substantial dysregulation of dopamine in these animals.
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Defended 05-19-2008
Research Competencies:

Behavioral Sensitization using Anymaze (Stoelting, Wood Dale, IL) Digital tracking software
In vivo microdialysis
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