



SCHOOL of
GRADUATE STUDIES
EAST TENNESSEE STATE UNIVERSITY

East Tennessee State University
Digital Commons @ East
Tennessee State University

Electronic Theses and Dissertations

Student Works

8-2008

Sex Differences in Nicotine-Conditioned Hyperactivity in a Model of Dopamine D2 Receptor Priming: Roles of Dopamine D2 and D3 Receptor Subtypes.

Ashley Brianna Sheppard
East Tennessee State University

Follow this and additional works at: <https://dc.etsu.edu/etd>

 Part of the [Hormones, Hormone Substitutes, and Hormone Antagonists Commons](#)

Recommended Citation

Sheppard, Ashley Brianna, "Sex Differences in Nicotine-Conditioned Hyperactivity in a Model of Dopamine D2 Receptor Priming: Roles of Dopamine D2 and D3 Receptor Subtypes." (2008). *Electronic Theses and Dissertations*. Paper 1978. <https://dc.etsu.edu/etd/1978>

This Thesis - Open Access is brought to you for free and open access by the Student Works at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact digilib@etsu.edu.

Sex Differences in Nicotine-conditioned Hyperactivity in a Model of Dopamine D2 Receptor
Priming: Roles of Dopamine D2 and D3 Receptor Subtypes

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

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

by
Ashley Brianna Sheppard
August 2008

Russell Brown, PhD., Chair
Michael Woodruff, PhD., Committee member
Otto Zinser, PhD., Committee member

Keywords: Conditioned Hyperactivity, Contextual Associations, D2 Priming, Antagonists,
Nicotine, Schizophrenia, Sensitization, Sex Differences, Smoking

ABSTRACT

Sex Differences in Nicotine-conditioned Hyperactivity in a Model of Dopamine D2 Receptor

Priming: Roles of Dopamine D2 and D3 Receptor Subtypes

by

Ashley Brianna Sheppard

The aim of this investigation was to determine the effect of a nicotine-conditioned context on locomotor hyperactivity in an animal model of D2-priming, and whether conditioned hyperactivity could be blocked by the D2 antagonist eticlopride or the D3 antagonist nafadotride. D2-primed male rats showed enhanced nicotine sensitization as evidenced by statistically significant differences in horizontal activity. D2-primed female rats administered nicotine demonstrated an increased hypoactive response after initial sensitization and increased stereotypy. Eticlopride and nafadotride blocked sensitization to nicotine in both D2-primed and non D2-primed males and females. Eticlopride blocked conditioned hyperactivity in females but not in males. D2-primed female rats administered nicotine demonstrated significantly higher conditioned-hyperactivity as compared to non D2-primed females and controls, and this increase was more effectively blocked by nafadotride as compared to eticlopride. These results suggest differential roles of the dopamine D2 and D3 receptors in both adolescent nicotine sensitization and conditioned activating effects of nicotine.

DEDICATION

This work is dedicated to Ben, Mom, Dad, and Tiff for being so supportive; Dr. Russ Brown for being a great advisor and also a great friend; and finally, the “weekend crew” who has been my family in our home away from home for the past two years: Zack, Daniel, Kim, and of course, Ben.

“Go confidently in the direction of your dreams. Live the life you have imagined.”

-Henry David Thoreau

ACKNOWLEDGEMENTS

This work was supported by a grant from the East Tennessee State University School of Graduate Studies. I would also like to acknowledge my thesis committee, Dr. Russ Brown, Dr. Mike Woodruff, and Dr. Otto Zinser, for their insightful and helpful suggestions in the preparation of this manuscript.

CONTENTS

| | Page |
|---|------|
| ABSTRACT | 2 |
| DEDICATION..... | 3 |
| ACKNOWLEDGEMENTS..... | 4 |
| LIST OF FIGURES | 8 |
| Chapter | |
| 1. INTRODUCTION | 9 |
| The Dopamine System | 10 |
| Rodent Models of Schizophrenia | 11 |
| Rationale for Use of Animal Models | 11 |
| Past Models of Schizophrenia: Dopamine Hyperactivity..... | 12 |
| Neonatal Quinpirole Model: A New Model of Schizophrenia..... | 14 |
| Behavioral Sensitization | 17 |
| Anatomy of the Nucleus Accumbens | 18 |
| Role of the Nucleus Accumbens in Behavioral Sensitization | 18 |
| Involvement of Dopamine Receptors in Nicotine Sensitization ... | 20 |
| Involvement of Non-dopaminergic Systems in Sensitization | 22 |
| Nicotine Conditioned Hyperactivity | 23 |
| Age Differences in the Effects of Nicotine on Behavior..... | 24 |
| Sex Differences in the Effects of Nicotine on Behavior | 26 |
| Research Questions Addressed in this Thesis | 29 |
| 2. METHODS..... | 31 |

| | |
|---|----|
| Subjects | 31 |
| Drug Dosage..... | 31 |
| Drug Treatment..... | 32 |
| Neonatal..... | 32 |
| Adolescent..... | 32 |
| Apparatus..... | 32 |
| Behavioral Testing Procedure | 33 |
| Research Design and Data Analysis | 34 |
| 3. RESULTS..... | 35 |
| Sex Differences in Nicotine Sensitization for Rats Administered | |
| Eticlopride..... | 35 |
| Initial Analyses..... | 35 |
| Horizontal Activity for Male Rats Administered Eticlopride | 36 |
| Horizontal Activity for Female Rats Administered Eticlopride..... | 37 |
| Sex Differences in Nicotine-Conditioned Hyperactivity for Rats Administered | |
| Eticlopride..... | 39 |
| Conditioned Hyperactivity for Male Rats Administered Eticlopride.... | 39 |
| Conditioned Hyperactivity for Female Rats Administered Eticlopride.. | 40 |
| Sex Differences in Nicotine Sensitization for Rats Administered | |
| Nafadotride..... | 41 |
| Initial Analyses | 41 |
| Horizontal Activity for Male Rats Administered Nafadotride..... | 42 |
| Horizontal Activity for Female Rats Administered Nafadotride..... | 43 |

| | |
|--|----|
| Sex Differences in Nicotine-Conditioned Hyperactivity for Rats Administered Nafadotride..... | 45 |
| Conditioned Hyperactivity for Male Rats Administered Nafadotride.. | 45 |
| Conditioned Hyperactivity for Female Rats Administered Nafadotride | 45 |
| 4. DISCUSSION..... | 47 |
| Nicotine Sensitization..... | 47 |
| Antagonism of Dopamine Receptor Subtypes..... | 49 |
| D2 Receptor..... | 49 |
| D3 Receptor..... | 49 |
| Dopamine Receptor Density in Adolescence | 50 |
| Alternative Mechanisms..... | 51 |
| Nicotine-Conditioned Hyperactivity..... | 51 |
| Clinical Relevance..... | 54 |
| Conclusion..... | 55 |
| REFERENCES | 56 |
| VITA | 79 |

LIST OF FIGURES

| Figure | Page |
|--|------|
| 1. Horizontal Activity for Males Administered Eticlopride..... | 37 |
| 2. Horizontal Activity for Females Administered Eticlopride..... | 38 |
| 3. Conditioned Hyperactivity for Males Administered Eticlopride..... | 40 |
| 4. Conditioned Hyperactivity for Females Administered Eticlopride..... | 41 |
| 5. Horizontal Activity for Males Administered Nafadotride..... | 43 |
| 6. Horizontal Activity for Females Administered Nafadotride..... | 44 |
| 7. Conditioned Hyperactivity for Males Administered Nafadotride..... | 45 |
| 8. Conditioned Hyperactivity for Females Administered Nafadotride..... | 46 |

CHAPTER 1

INTRODUCTION

Smoking is a convenient and legal form of drug self-administration that often begins in adolescence. Addiction to nicotine, the psychomotor stimulant drug found in cigarettes, often results (Benowitz, 1988; Faraday, Elliott, Phillips, & Grunberg, 2003; Jarvis, 2004). Nicotine has positive reinforcing effects that typically become associated with environmental and contextual cues (Bevins & Palmatier, 2003; Bevins, Eures, & Besheer, 2004; Jarvis; Lazev, Herzog, & Brandon, 1999). After many pairings of smoking with a particular environment or within a specific context, the context in which the individual smokes appears to elicit strong cigarette cravings followed by smoking behavior (Bevins & Palmatier; Brandon, Piasecki, Quinn, & Baker as cited in Lazev et al., 1999; Jarvis). A rise in smoking-related health problems, evidenced by lung cancer surpassing breast cancer as the leading type of cancer in women in the United States, suggests that women in general are more sensitive to the effects of nicotine, have greater difficulty quitting, and use tobacco more frequently than men (Grunberg, Winders, & Wewers, 1991; Kandel, Warner, & Kessler as cited in Harrod et al., 2004; Waldron, 1991).

The schizophrenic population in particular exhibits especially high rates of smoking which is nearly three times that of the general population (Kelly & McCreadle, 1999). According to the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000), schizophrenia is a psychotic disorder in which patients experience symptoms such as delusions, hallucinations, disorganized speech, catatonic motor behavior, and negative affect. The disorder usually has an age of onset between the late teens and mid-30s (American Psychiatric Association). Individuals suffering from schizophrenia exhibit impairments in many cognitive functions such as memory, attention, and

psychomotor abilities (American Psychiatric Association). The high incidence of smoking among these patients raises the possibility of an underlying neurochemical mechanism involving nicotine in which the midbrain dopaminergic system, also referred to as the mesocorticolimbic pathway, has been implicated (Dalack, Healy, & Meador-Woodruff, 1998).

The Dopamine System

Dopamine, a catecholamine neurotransmitter that contains an amine group and a catechol ring, is synthesized from the amino acid tyrosine obtained from dietary protein (Kalat, 2004). Dopamine is a modulatory neurotransmitter that acts via two families of G-protein-coupled receptors that are divided into these two families based on similar pharmacological profiles towards dopamine agonists and antagonists (Cooper, Bloom, & Roth as cited in Kostrzewa, 1995; Missale, Nash, Robinson, Jaber, & Caron, 1998). The dopamine D1-like receptor family consists of the D1 and D5 receptor types and the dopamine D2-like receptor family consists of D2, D3 and D4 receptor types (Kostrzewa, 1995). Overactivity of the dopamine system has been implicated as playing a primary role in schizophrenia, obsessive-compulsive disorder (OCD), and bipolar disorder (Kostrzewa). Also, genetic polymorphisms of the D2 and D4 receptors have been reported in attention-deficit hyperactivity disorder (ADHD) (Bobb, Castellanos, Addington, & Rapoport, 2005).

Behavioral changes resulting from the pharmacological induction of increases in dopaminergic activity in laboratory animals have been shown to produce symptoms similar to those observed in schizophrenic patients (Braff & Geyer, 1990). For example, studies from our laboratory have shown deficits in prepulse inhibition (PPI), a deficit commonly seen in schizophrenia (Adler, Freedman, Ross, Olincy, & Walso, 1998). This effect is hypothesized to result from increased dopamine D2 receptor supersensitivity as a consequence of neonatal

quinpirole treatment (Smith, Perna, & Brown, unpublished data). Interestingly, animal studies by Brown et al. (Brown & Kolb, 2001; Brown, Thompson, Thompson, Thacker, Ward, et al., 2004) have also shown that adulthood nicotine treatment can improve or alleviate cognitive deficits produced by neonatal quinpirole treatment. This suggests that schizophrenics may self-medicate through smoking in an attempt to assuage cognitive deficiencies produced by overactive dopamine function inherent to the disorder, although the mechanism through which nicotine works has yet to be delineated (Kumari & Postma, 2005).

It has also been hypothesized that gliosis leading to a decrease in prefrontal dopamine activity subsequently results in an increase in dopamine D2 receptor sensitivity and mesocorticolimbic dopamine activity (Angelucci, Mathe, & Aloe, 2004; Davis, Kahn, Ko, & Davidson, 1991). Mesocorticolimbic dopamine neuron hyperactivity, coinciding with prefrontal dopamine hypoactivity, has been associated with cognitive deficits observed in schizophrenia (Davis et al.; Elvevag & Goldberg, 2000; Mackay et al., 1982). Additionally, increases in dopaminergic activity have been shown to depress cholinergic functioning, and cholinergic function has been shown to play a major role in cognition (Elvevag & Goldberg). Studies from our laboratory (Brown, Thompson, Thompson, Thacker, Ward, et al., 2004; Brown, Perna, Schaffer, & Williams, 2006) as well as Day and Fibiger (1992) have shown that activation of dopamine D2 receptors depresses acetylcholine function in the hippocampus and produces cognitive impairment. Thus, it appears that increases in dopaminergic function can result in changes that alter other neurotransmitter systems with behavioral consequences.

Rodent Models of Schizophrenia

Rationale for Use of Animal Models

Animal models are valuable tools for studying aspects of diseases. Although there are limitations, such as the inability to study the long-term progression of a disease, animal models can be used as a means to elucidate underlying mechanisms and develop effective treatments for neurological disorders (Woodruff & Baisden, 1994). Another important aspect of animal models is that although it may be difficult to completely replicate a disorder in an animal, modeling even one aspect of that disorder can be very useful in discovering treatments. For example, Alzheimer's disease results in neurodegeneration of all neurotransmitter systems in the brain; however, drug treatments designed to enhance cholinergic activity were discovered as the result, at least in part, on animal models of cognitive dysfunction (Yamada & Nabeshima, 2000). Relevant to this proposal, several rodent models of schizophrenia that used mesolimbic dopamine hyperactivity as the neural basis of the disease have proven useful in understanding behavioral and biochemical aspects of the disorder.

Past Models of Schizophrenia: Dopamine Hyperactivity

Several past rodent models of schizophrenia have used acute administration of drugs such as apomorphine (Lacroix, Broersen, Feldon, & Weiner, 2000), amphetamine (Kokkinidis & Anisman, 1980), methamphetamine and cocaine (Yamamoto, 1997) that stimulate the dopamine system. A recently developed model of schizophrenia in which rats were administered amphetamine i.p. 3 days per week for 5 consecutive weeks has also been shown to create dopamine receptor supersensitivity as evidenced by amphetamine-induced locomotor activity and disruption in sensorimotor gating in animals tested using the PPI paradigm (Tenn, Fletcher, & Kapur, 2003; Tenn, Kapur, & Fletcher, 2005). Additionally, Lacroix et al. (2000) infused the

indirect dopaminergic receptor agonist apomorphine into the prefrontal cortex which caused a disruption in prepulse inhibition, a behavioral manifestation of schizophrenia. Although these models have been beneficial in studying schizophrenia, acute administration of dopaminergic agonists does not represent the long-term dopamine dysfunction seen in schizophrenic patients.

Other models have used acute administration of phencyclidine (PCP), an NMDA receptor antagonist, to mimic NMDA receptor hypofunction that is known to occur in schizophrenia (Heresco-Levy, Silipo, & Javitt, 1996; Millan, 2002, 2005). Noncompetitive blockade of NMDA receptors using PCP produces both positive and negative symptoms of schizophrenia in normal subjects (Heresco-Levy et al.). Studies using this model have shown prepulse inhibition deficits and social withdrawal consistent with schizophrenia (Sams-Dodd, 1997). Additional studies have shown alleviation of both positive and negative symptoms of schizophrenia using NMDA agonists such as glycine and D-serine, suggesting NMDA receptors as targets for potential therapies (Heresco-Levy et al.).

Lipska and Weinberger (2002) have developed a rodent model of schizophrenia through ablation of the ventral hippocampus in 7-day-old rats. The rationale for this model is based on known hippocampal neuropathology present in schizophrenia including decreased hippocampal volume and increased size of the lateral ventricles (Harrison & Eastwood, 2001). Findings from this model have shown reductions of dopamine transporter (DAT) mRNA in the VTA and substantia nigra in adult rats (Lipska, Lerman, Khaing, & Weinberger, 2003) as well as several behavioral deficits seen in schizophrenia including increased locomotor activity and cognitive impairments (Chambers, Moore, McEvoy, & Levin, 1996). This particular model is challenging due to unavoidable damage to cortical and some subcortical areas as a result of lesioning the hippocampus at the early age at which the procedure must be performed. Also, changes in

hippocampal synaptic connectivity, but not cell death, have been observed in schizophrenic patients postmortem, creating another weakness for this particular model (Harrison & Eastwood).

Neonatal Quinpirole Model: A New Model of Schizophrenia

Quinpirole, a dopamine D2/D3 receptor agonist, administered to rats during the neonatal period has been shown to produce long-term dopamine D2 receptor supersensitivity, also known as 'D2 receptor priming'(Kostrzewa, 1995; Kostrzewa & Brus, 1991; Kostrzewa, Hamdi, & Kostrzewa, 1990). Priming of the D2 receptor refers to increased physiological, biochemical, or behavioral responses of dopamine D2 receptors to dopamine agonists and is thought to account for altered drug-induced behavioral responses in several neurological disorders including schizophrenia, bipolar disorder, and attention-deficit hyperactivity disorder (ADHD) (Bobb, Castellanos, Addington, & Rapoport, 2005; Kostrzewa; Rosa-Neto et al., 2005; Versiani, 2006). Dopamine D2 receptor priming has been shown to alter overall brain function through influences on other neurotransmitter systems, including the acetylcholinergic system (Brown et al., 2004; Brown, Perna, Schaefer, & Williams, 2006).

Similarities have been observed between the neonatal quinpirole model of schizophrenia and data from the human schizophrenia literature. For example, amphetamine administration to adult rats neonatally treated with quinpirole produce a robust increase in dopamine release in the striatum (Nowak, Brus, & Kostrzewa, 2001). Studies using magnetic resonance imaging (MRI) and positron emission tomography (SPECT) imaging have shown that amphetamine administration also produces a large increase in dopamine release in the striatum of schizophrenics (Lavalaye et al., 2001; Soares & Innis, 1999).

Secondly, neonatal quinpirole treatments have been shown to produce deficits in auditory sensorimotor gating using prepulse inhibition (PPI). PPI of the startle response refers to attenuation in response to a strong stimulus (pulse) if this is preceded shortly by a weak non-startling stimulus (prepulse). PPI provides a simple operational measure of sensorimotor gating that is often disrupted in schizophrenia (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Kumari & Sharma, 2002). Our laboratory has shown that adult rats that received neonatal quinpirole treatment demonstrated PPI deficits as compared to controls using different prepulse auditory intensities (73, 76, and 82 dB) and different intervals between the prepulse and pulse (50, 100, and 150ms) (Maple, unpublished data).

Third, neonatal quinpirole treatments have been shown to produce long-term cognitive impairment (Brown, Gass, & Kostrzewa, 2002; Brown, Thompson, Thompson, Thacker, Ward, et al., 2004). It has been well-documented that severe cognitive impairments are present in schizophrenia, and it has been suggested that cognitive impairment is a core feature of the disorder (Adler et al., 1998; Adler, Freedman, Ross, Olincy, & Walso, 1999; Elvevag & Goldberg, 2000). Cognitive deficits have also been hypothesized to be associated with sensorimotor gating in schizophrenics (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001).

Fourth, our laboratory has very recently shown that chronic treatment with the atypical antipsychotic olanzapine, or Zyprexa, given twice daily in adulthood alleviated cognitive deficits produced by neonatal quinpirole treatment (Thacker et al., 2006). Importantly, this treatment also alleviated the significant increase in yawning produced by rats neonatally treated with quinpirole to control levels, essentially reversing the D2-priming effect. These data demonstrate that not only is D2-priming likely primarily responsible for these behavioral effects, but that antipsychotic treatments are effective in alleviating these effects. Additionally, *in vitro* analyses

showed that significant decreases in nerve growth factor (NGF) in the hippocampus produced by neonatal quinpirole were reversed by olanzapine in brain tissue that was not taken until after an 8-day olanzapine washout (Thacker et al.). However, expression of proteins was affected only in the hippocampus and not in areas known to be important in cognitive functioning suggesting a different mechanism for apparent alleviation of cognitive deficits in the Morris Water Maze task (Brown, Perna, Maple, Wilson, & Miller, 2008).

Fifth, neonatal quinpirole treatments have been shown to alter overall brain function through influences on other neurotransmitter systems, including the acetylcholinergic system, (Brown et al., 2004; Brown, Perna, Schaefer, & Williams, 2006) and produce neurochemical abnormalities in adulthood that are similar to observations made in human schizophrenics. Results from this laboratory have shown that neonatal quinpirole treatment produced a 36% decrease in choline acetyltransferase (ChAT) and a significant decrease in nerve growth factor (NGF) expression in the hippocampus compared to saline controls in both early postweaning and adult rats (Brown et al., 2004; Brown, Thompson, Click, Thacker, & Perna, 2005; Brown, Perna, Maple, Wilson, & Miller, 2008). Research in non-medicated human schizophrenics have demonstrated a decrease in overall NGF expression, which has been suggested to account for neurodevelopmental abnormalities seen in schizophrenia (Aloe, Iannitelli, Angelucci, Bersani, & Fiore, 2000; Parikh, Evans, Kahn, & Mahadik, 2003).

Finally, PCR analyses from our laboratory have shown a significant decrease in the genetic expression of alpha7 nicotinic receptors in the hippocampus of D2-primed animals (Brown et al., unpublished data). Reduced alpha7 expression is consistent with studies in human schizophrenic patients that have shown a reduction in availability in the alpha7 nicotinic receptor subunit gene (Leonard et al., 2002). Recently our lab has also shown decreases in RGS-9 protein

expression (Maple, Perna, Parlaman, Stanwood, & Brown, 2007) in agreement with Seeman et al. (2007) who have shown reductions in RGS9-2 mRNA expression in hippocampus tissue of schizophrenics.

Therefore, it appears there are several similarities between the D2 receptor priming model and schizophrenia, and this model may be valid for the study of this disorder. However, further investigation of the effects of D2 priming on the serotonergic system, and the presence of NMDA receptor hypofunction is required in order to further validate the neonatal quinpirole model of schizophrenia.

Behavioral Sensitization

Behavioral sensitization is defined as an increase in behavioral response to repeated presentations of a stimulus (Kelley & Rowan, 2004; Wise & Bozarth, 1987). Locomotor activity is defined as horizontal movement and vertical rearing and is thought to result from activation of mesolimbic dopamine projections from the ventral tegmentum to the nucleus accumbens during psychomotor drug stimulation of the reward pathway, although other neurotransmitters are undoubtedly involved as well (Clarke, Fu, Jakubovic, & Fibiger, 1988; Kalivas & Stewart, 1991; Wise & Bozarth). Several drugs in the psychostimulant class, including amphetamine, cocaine, and nicotine, have been shown to increase dopamine function and also produce locomotor sensitization (Booze, Welch, Wood, Billings, Apple, & Mactutus, 1999; Chiang, Chen, & Chen, 2003; Kosowski & Liljequist, 2005; Kuczenski & Segal, 2001; Perna, in press; Segal & Kuczenski, 1992; Tenn, Kapur, & Fletcher, 2005). Additionally, blockade of dopamine D1 and D2 receptors through pretreatment with a D1 or D2 antagonist such as SCH 23390 or eticlopride have been shown to block sensitization to psychostimulants (Schindler & Carmona, 2002). Further, ablation of areas rich in dopaminergic cell bodies in the ventral tegmental area or

dopaminergic terminals in the nucleus accumbens has also been shown to be effective in blocking locomotor sensitization to psychostimulant drugs (Clarke et al., 1988). Thus, it appears that the dopamine system plays a crucial role in locomotor sensitization to psychostimulants.

Anatomy of the Nucleus Accumbens

The primary brain pathway involved in drug addiction appears to be the mesocorticolimbic pathway that originates from dopaminergic cell bodies in the ventral tegmental area (VTA) of the midbrain and projects to the nucleus accumbens (NAcc), amygdala, hippocampus, bed nucleus of the stria terminalis, and prefrontal cortex (Kumari & Postman, 2005; Vetulani, 2001). The nucleus accumbens has been shown to be the center of primary drug reward in the brain and has been histologically defined in two parts: the core and the shell. Based on histological analysis, the shell of the nucleus accumbens (AccSh) has been proposed to be an extension of the amygdala, the emotional center of the brain that is thought to play an important role in associative learning, particularly in situations involving rewarding and emotional behaviors (Everitt, Morris, O'Brien, & Robbins, 1991; Meredith, Callen, & Scheuer, 2002). The core sends projections to the substantia nigra and other areas associated with motor function such as the putamen, caudate, globus pallidus, as well as the frontal cortex (Balfour, Wright, Benwell, & Birrell, 2000; Mogenson & Yang, 1991). Increased dopamine transmission in the nucleus accumbens shell during or immediately after drug administration is thought to account for the reinforcing properties of drug use that facilitates addiction by consolidating associative learning neural traces via D1 receptors (Di Chiara et al., 2004).

Role of the Nucleus Accumbens in Behavioral Sensitization

Increased locomotor activity after treatment with psychostimulants has been shown to result from stimulation of dopaminergic neurons located in the nucleus accumbens core (Balfour

et al., 2000; Imperato & Di Chiara as cited in Joseph, Datta, & Young, 2003; Pontieri, Tanda, Orzi, & Di Chiara, 1996). According to the psychomotor theory of drug addiction proposed by Wise and Bozarth (1987), locomotor activity can be used to predict the reinforcing effects and addictive liability of drugs because both effects stem from a common mechanism involving the nucleus accumbens that is also responsible for reinforcement associated with natural rewards such as food and water. Natural rewards stimulate aversive centers through a feedback mechanism in order to control reward-seeking behaviors. However, artificial chemical rewards such as drugs of abuse stimulate the reward pathway but are not restrained by the controlling feedback mechanism possibly producing addiction (Vetulani, 2001).

Drugs in the psychostimulant class, including nicotine, have been shown to have strong associative properties that likely make them susceptible to addiction. For example, Pontieri et al. (1996) used microdialysis, a technique for collecting neurotransmitter samples by perfusion of artificial cerebrospinal fluid through a particular brain region using a specialized probe (Joseph et al., 2003), to show dose-dependent increases in dopamine transmission in the nucleus accumbens of rodents that were administered intravenous (i.v.) nicotine. Nicotine was administered via an i.v. pulse in an attempt to approximate the pharmacokinetics of inhaled nicotine from tobacco smoke. Pontieri et al. suggest that positive motivational states induced by NAcc dopamine transmission may facilitate the association of contextual stimuli with the psychostimulant effects of nicotine to acquire incentive properties through the process of motivational learning.

Studies have shown an increase in dopamine release over subsequent administration of a range of psychostimulants including amphetamine, cocaine, and methamphetamine (Akimoto, Hamamura, Kazahaya, Akiyama, & Otsuki, 1990; Kalivas & Duffy, 1990; 1993; Wolf, White,

Nassar, Brooderson, & Khansa, 1993). Behavioral sensitization is thought to result from this increased dopamine release by neurons located in the VTA which creates dopamine overflow in the nucleus accumbens core (Kalivas & Stewart, 1991). The NAcc core sends retroactive projections to the midbrain motor centers, including substantia nigra, medial subthalamic nucleus, and pedunculopontine motor region, resulting in increased locomotor activity (Pierce & Kalivas, 1997).

Involvement of Dopamine Receptors in Nicotine Sensitization

It has been suggested that decreased inhibition of neurons controlled by presynaptic dopamine D3 autoreceptors or increased postsynaptic D1 or D2 receptor sensitivity to dopamine in the nucleus accumbens and striatum may play a role in the development of behavioral sensitization and are associated with the processes underlying addiction (Balfour et al., 2000; Chiang, Chen, & Chen, 2003; Le Foll, Diaz, & Sokoloff, 2003). Behavioral sensitization induced by psychostimulant drugs such as nicotine may specifically reflect stimulation of dopamine receptors within the core of the nucleus accumbens. However, results from a study by Le Foll, Diaz, et al. (2003) showed increases in dopamine D3 receptor binding and mRNA expression in the nucleus accumbens shell, but not core, after subchronic nicotine administration. In addition to increased D3 receptor expression, studies have shown increases in dopamine transporters in response to repeated nicotine administration (Harrod et al., 2004). Interestingly, studies to date have failed to demonstrate changes in D1 or D2 receptor expression or sensitivity after repeated nicotine administration (Harrod et al.; Le Foll, Diaz, et al.).

Dopamine D3 receptors have been shown to have greater occupancy for dopamine as compared to D1 and D2 receptor subtypes (Richtand, Goldsmith, Nolan, & Berger, 2001). It has been hypothesized that locomotor activity in rodents is mediated by the opposing actions of

D1/D2 receptor activation and D3 receptor inhibition, and that rapid D3 receptor tolerance due to increased affinity for dopamine and D3 receptor down-regulation after chronic psychostimulant administration leads to observed increases in locomotor activity (Richtand, Goldsmith, Nolan, & Berger, 2001; Richtand et al., 2000). D3 receptor antagonism has been shown to block sensitization to amphetamine (Chiang, Chen, & Chen, 2003; Richtand et al., 2000) providing support for this hypothesis. However, Filip, Papla, and Czepeil (2002) observed increases in the expression of sensitization to cocaine after administration of the D3 receptor antagonist nafadotride on the 10th day of drug administration. Another study showed that although sensitization was observed, there were no significant differences in locomotor activity between wildtype and D3-receptor knockout mice after repeated cocaine administration, suggesting that D3-receptors do not play an important role in the expression of behavioral sensitization (Betancur et al., 2001). These results may reflect species-specific neurochemical differences between rats and mice or the development of a compensatory mechanism of D3 receptor deficient mice to expression of sensitization. Therefore, involvement of the D3 receptor in sensitization to different psychostimulants warrants further investigation in both species.

In a study by Le Foll, Schwartz, and Sokoloff (2003), D3 antagonism blocked the conditioning effects of nicotine in rats that had nicotine repeatedly paired with a particular environment. In another study utilizing the conditioned-place preference paradigm, Le Foll, Sokoloff, Stark, and Goldberg (2005) showed that the selective D3 receptor antagonist ST 198 blocked the expression of nicotine-conditioned place preference without affecting locomotor activity (Le Foll, Sokoloff, et al.). These results suggest that D3 receptors are selectively involved in the associative effects of nicotine but may not play a role in the locomotor activating effects of the drug (Le Foll, Schwartz, et al.; Le Foll, Sokoloff, et al.).

The expression of D3 receptors and behavioral sensitization is thought to result from changes in striatal and accumbal connectivity by repeated nicotine-administration triggered release of brain-derived neurotrophic factor (BDNF) originating from cortico-striatal neurons (Guillin et al., 2001; Le Foll, Diaz, et al., 2003). Guillin et al. (2001) have suggested that repeated use of addictive drugs alters BDNF expression that subsequently influences dopamine responsiveness in areas associated with environmental cues and context, such as the amygdala, inducing drug-conditioned responses to contextual stimuli. In line with this hypothesis, LeFoll, Diaz, et al. have also shown increased D3 receptor expression in the nucleus accumbens after repeated conditioning of nicotine to an environmental context. Additional studies are needed to determine the role of D3 receptors and their interaction with BDNF in drug addiction.

Involvement of Non-Dopaminergic Systems in Nicotine Sensitization

Balfour et al. (2000) suggest that projections in the shell of the nucleus accumbens exhibit burst firing that is dependent upon N-methyl-D-aspartate (NMDA) receptor stimulation by acute nicotine administration and is important for the initiation of behavioral sensitization. However, chronic nicotine administration is required to stimulate burst firing in core projections. Support for this hypothesis was demonstrated in several recent studies. Results from these studies showed that NMDA receptor antagonists inhibited nucleus accumbens core dopamine overflow in sensitized animals that were pretreated with nicotine. This group did not show significant changes in the augmented locomotor response, suggesting a major role of post synaptic dopamine receptors in the mediation of nicotine sensitization (Balfour, Birrell, Moran, & Benwell, 1996; Birrell & Balfour, 1998; Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994; Suemaru, Gomita, Furunu, & Araki, 1993).

Nicotine Conditioned Hyperactivity

Behavioral sensitization can be achieved by using classical Pavlovian conditioning in which a previously neutral stimulus, such as a particular environment, can acquire rewarding properties through learned association. This occurs when a biologically rewarding stimulus, such as nicotine, is temporally paired with an environmental context. Drug-associated conditioned stimuli may also acquire the ability to elicit responses related to cravings, withdrawal, and drug seeking behavior (Robinson, & Berridge, 1993; Siegel as cited in Bevins & Palmatier, 2003).

Bevins and Palmatier (2003) used a locomotor conditioning paradigm in which nicotine was repeatedly administered and reliably paired with a locomotor arena every day for a total of 8 conditioning days. Contextual conditioning was followed by a drug-free trial in which animals were given a saline injection and placed into the same locomotor arena that had been reliably paired with nicotine. Results from this study showed significant increases in locomotor activity in drug-context paired groups as compared to saline-context paired groups during the postconditioning drug-free test, demonstrating the ability of an environmental context stimulus to evoke nicotine-conditioned hyperactivity.

Nicotine conditioning to a particular context elicits locomotor hyperactivity that has been hypothesized to result from positive reinforcement and, in theory, should also elicit increased dopamine release. Although a significant increase in activity could result from other changes produced by the drug, such as nicotine-induced nicotinic receptor upregulation, studies have shown that dopamine may play an important role in nicotine conditioned hyperactivity. This hypothesis is supported by the ability of D2 antagonists such as haloperidol to interfere with conditioned stimulus control of sensitization (Kalivas & Stewart, 1991) and the ability of the selective D3 antagonist SB-277011A to decrease nicotine-induced conditioned locomotor

activity in rats (Pak et al., 2006). Bevins, Besheer, and Pickett (2001) have shown that administration of the selective D1 antagonist SCH-23390 during the post nicotine drug-free trial prevented the expression of a context-nicotine association. In a subsequent study, administration of SCH-23390 during the conditioning phase did not affect the acquisition of a nicotine-environment association suggesting a greater role of the D1 receptor in the expression, as opposed to acquisition, of conditioned hyperactive behavior induced by nicotine (Bevins et al.).

Increases in nucleus accumbens dopamine release during drug-free testing would suggest that schizophrenic patients may experience greater drug reinforcement when exposed to drug-associated cues than a normal individual due to further activity in an already hyperactive dopaminergic system. Whether this increased locomotor activity is a product of an overabundance of dopamine or reflects an increase in dopamine receptor sensitivity is yet to be determined. Although dopamine appears to play an important role, the roles of other neurotransmitter systems should also be evaluated.

Age Differences in the Effects of Nicotine on Behavior

Individuals who begin smoking at an early age have increased difficulty quitting as adults (Jarvis, 2006). Faraday et al. (2003) suggest that this is due to differences in acute and long-term effects of nicotine. Studies have shown that both male and female adolescent rats exposed to nicotine demonstrated increased activity compared to saline control animals without drug administration, whereas adult rats administered the same doses showed activity levels comparable to saline-controls (Elliot, Faraday, Phillips, & Grunberg, 2005; Faraday, Elliot, Phillips, & Grunberg, 2003). Adolescent rats also showed less sensitivity to nicotine's initial hypoactivity effects and adolescents that were initially exposed to nicotine demonstrated an

overall increase in activity when reexposed to the drug as adults suggesting that early exposure may alter adulthood response to nicotine (Elliot et al.; Faraday et al.).

In a recent study from our laboratory, dopamine D2-primed adolescent rats demonstrated a more robust sensitization to nicotine as compared to adult rats (Perna, under review). Nicotine sensitization was more robust in female subjects neonatally treated with saline as compared to male subjects that received the same neonatal treatment. This is consistent with past findings by Booze et al. (Booze et al., 1999; Harrod et al., 2004).

There are several possible mechanisms for age differences in reactions to nicotine. It has been hypothesized that there may be different rates of metabolizing nicotine between adults and adolescents (Faraday et al., 2003). However, there have been no significant differences in nicotine metabolism mediated by CYP2A6, an enzyme important for the clearance of nicotine, in adolescents as compared to adults (Hukkanen, Jacob, & Benowitz, 2005). Lapin et al. (1987) also failed to find sustained changes in metabolism after both acute and sustained nicotine exposure. Additionally, age differences in nicotinic acetylcholine receptor distribution, density, and affinity may also partly account for age differences in nicotine reactivity based on the regulation of both decreased and increased activity by central nicotinic cholinergic systems (Di Chiara as cited in Faraday et al., 2003; Stolerman, Garcha, & Mirza, 1995) Faraday et al. (2003) suggest that a difference in nicotinic receptor subpopulations may exist with lower doses activating the activity-decreasing subpopulation to a lesser extent in adolescents as compared to adult rats.

Rate differences in receptor up-regulation and desensitization in response to drug administration have been observed as adolescent D3 receptor levels at only 40% of adult levels (Faraday et al. 2003; Shram, Funk, Li, & Le, 2006). Although speculative, this suggests

schizophrenic patients who begin smoking during adolescence may have a more difficult time quitting due to the decrease in negative responding to initial nicotine exposure possibly resulting from decreased prevalence of the D3 receptors coupled with the potential cognitive deficit alleviating capacity of nicotine. Differences in the rates of receptor up-regulation and desensitization in response to nicotine administration may be especially important when examining nicotine addiction in schizophrenia due to dopamine receptor dysfunction thought to underlie the disorder.

Sex Differences in the Effects of Nicotine on Behavior

Although no sex differences in the initial response to nicotine have been reported, female rats exhibit increased locomotor activity, greater sensitivity to lower doses, as well as an increased number of the dopamine transporter (DAT) in both the shell and core of the NAcc after chronic nicotine administration as compared to males (Booze et al., 1999; Elliot et al., 2005; Faraday et al., 2003; Harrod et al., 2004; Kanyt, Stolerman, Chandler, Saigusa, & Pogun, 1999; Perna, manuscript in preparation). Studies have shown increased levels of dopamine transporter in female rats as compared to males resulting in increased synaptic availability of dopamine that may account for, in part, for sex differences in locomotor activity (Booze et al., 1999; Harrod et al., 2004). Studies have also shown that female rats do not exhibit hyperactive effects in the absence of nicotine seen in males given the same doses (Elliot et al.; Faraday et al.). Sex differences in dopamine D1 and D2 receptor sensitivity in response to both agonists and antagonists after psychostimulant administration suggests that both receptors are important in the locomotor activating effects observed in behavioral sensitization (Frantz & Van Hartesveldt, 1999; Schindler & Carmona, 2002). Differential expression of D3 receptors may also partially explain sex differences in locomotor activity levels. It is possible that female rats exhibit higher

activity levels due to a decrease in D3-regulated inhibition of activity. This hypothesis is supported by autoradiography studies that have shown decreased numbers of D3 receptors in the NAcc in female rats as compared to males after repeated intravenous nicotine administration (Harrod et al., 2004).

Schindler and Carmona (2002) found a significant antagonistic effect of SCH 23390 and increased locomotor activating effects of the dopamine uptake inhibitor GBR 12909 and the D1 agonist SKF 92958 in female rats as compared to males after cocaine administration. These results suggest increased dopamine D1 receptor sensitivity in females. The authors also found that male rats were more sensitive to the activity depressing effects of quinpirole suggesting that D2 receptor function may possibly account for the lower activity of males as compared to female rats after psychostimulant drug administration (Schindler & Carmona).

Studies from this laboratory (Brown, Thompson, Click, Best, Thacker, & Perna, 2005; Brown, Perna, Schaefer, & Williams, 2006) have shown a three-fold increase in yawning, a D2-mediated behavior, and deficits on a match-to-place Morris Water Task (MWT) in males neonatally treated with quinpirole as compared to female rats suggesting further sex differences in dopamine receptor sensitivity. Acute administration of the dopamine D2 antagonist eticlopride before place-version MWT training to animals neonatally treated with saline produced deficits in both male and female rats with males also showing deficits in a match-to-place MWT suggesting a greater importance of the D2 receptor in cognitive performance in male versus female rats (Brown, Thompson, et al.; Brown, Perna, et al.).

It is possible that sex differences observed in behavioral sensitization may be due to sex differences in nicotine distribution, metabolism, (Kyerematen, Owens, Chattopadhyay, deBethizy, & Vesell, 1988) and pharmacokinetics as evidenced by higher nicotine arterial plasma

levels in female rats chronically administered nicotine (Harrod, Booze, & Mactutus, 2007). It is thought that sex-dependent differences observed in behavioral sensitization result in part from modulation of striatal dopamine release by gonadal hormones (Booze et al., 1999; Dluzen & Anderson, 1997; Harrod et al., 2004; Kanyt et al., 1999). Becker (1999) has shown that estrogen down-regulates dopamine D2 autoreceptors and causes decreased firing of projections that synapse on gamma-aminobutyric acid (GABA) –B receptor subtypes found on dopamine receptor terminals resulting in enhanced striatal dopamine release. This enhancement of dopamine release is thought to result in enhanced behavioral responses including locomotor hyperactivity as a result of amphetamine stimulation, sensorimotor efficiency, and pacing during sexual behavior (Becker, 1999). Dluzen and Anderson (1997) reported greater dopamine output in tissue from castrated females treated with estrogen as compared to both male rats and female castrated animals not treated with estrogen. The authors also showed a decrease in striatal dopamine release in castrated males treated with estrogen demonstrating sex-dependent bidirectional effects of estrogen (Dluzen & Anderson).

Studies have shown estrous cycle-dependent increases in amphetamine-induced release of striatal dopamine as well as influences on sensorimotor functions and stereotypic behavior (Becker & Cha, 1989; Becker, Snyder, Miller, Westgate, & Jenuwine, 1987). Estrous cycle effects were not observed in nicotine self-administration behavior nor did estrogen appear to have a significant effect on nicotine-induced locomotor hyperactivity in female rats (Donny et al., 2000; Kuo et al., 1999). However, Franklin et al. (2004) have demonstrated significantly less cue-induced cravings in women in the follicular phase of the estrous cycle as compared to women in luteal phase or men suggesting an influence of sex hormones in response to nicotine or at least in response to nicotine-associated cues. Further studies are needed to determine the

extent, if any, that estrous cycle hormones affect behavioral responses to nicotine and nicotine-associated cues and whether these responses are altered by dopamine D2 receptor supersensitivity.

Research Questions Addressed in this Thesis

The aim of this study was to examine the following:

1) Analyze the effects of the dopamine D2 and D3 receptor subtype antagonists eticlopride and nafadotride, respectively, on the ability of a nicotine-conditioned context to elicit locomotor activity in D2-primed and non-D2-primed adolescent rats. It was expected that the D2 antagonist eticlopride (Bevins & Palmatier, 2002), but not the D3 antagonist nafadotride, would block induction of nicotine sensitization. This prediction was guided by Murray and Bevins (2007) who reported that relatively high doses of nafadotride are required to inhibit locomotor activity and that this inhibition is probably the result of loss of D3 receptor selectivity. However, based on the findings that nafadotride blocks the association of nicotine to the locomotor arena, it was expected that nafadotride, but not eticlopride, would block nicotine-conditioned hyperactivity during the drug-free trial (Bevins & Palmatier; Le Foll, Diaz, et al., 2003; Le Foll, Schwartz, et al., 2003).

2) Compare nicotine-conditioned hyperactivity and locomotor sensitization in D2-primed and non-D2-primed adolescent rats. A nicotine-conditioned environment was predicted to elicit locomotor hyperactivity as supported by past evidence from Bevins and Palmatier (2002) and that rats neonatally treated with quinpirole would show greater levels of hyperactivity as compared to controls.

3) Analyze sex differences in nicotine-conditioned hyperactivity in both D2-primed and non-D2-primed adolescent male and female rats. It was predicted that female rats would

demonstrate higher activity levels as a result of sensitization to nicotine in the conditioned-hyperactivity paradigm. This hypothesis was supported by past evidence that has shown females demonstrate a more robust locomotor response to nicotine (Booze et al., 1999; Elliot et al., 2005; Faraday et al., 2003; Harrod et al., 2004; Kanyt et al., 1999; Perna, in press). This increased locomotor response would be further accentuated in D2-primed rats and greater activity will be observed in females versus males.

4) Analyze sex differences in the effects of dopamine D2 and D3 antagonists eticlopride and nafadotride, respectively, on nicotine-conditioned hyperactivity in both D2-primed and non-D2-primed adolescent male and female rats. It was predicted that female rats would show higher levels of locomotor activity as compared to males in response to administration of dopamine D2 and D3 receptor antagonists. These hypotheses were based on results indicating increased dopamine D1 receptor sensitivity to the activating effects of D1 receptor agonists and decreased sensitivity to activity depressing effects of D2 receptor agonists in female rats as compared to males (Schindler & Carmona, 2002). Inhibition of the D2 receptor would lead to a greater decrease in activity levels in male rats as compared to females. The expected result was that females would exhibit higher activity levels in response to the dopamine D3 receptor antagonist. This may possibly result from a decrease in D3-regulated inhibition of activity based on findings that female rats had fewer D3 receptors in the NAcc after repeated intravenous nicotine administration as compared to males (Harrod et al., 2004).

CHAPTER 2

METHODS

Subjects

Ninety-eight offspring of Sprague-Dawley rats ordered from Harlan, Inc. (Indianapolis, IN) were used in both experiments. The day of birth was counted as postnatal day zero (P0) and pups were weaned from the dam at P22. Animals were housed in a climate-controlled vivarium maintained on a 12 h light: 12 h dark cycle and food and water were available ad libitum. All procedures used in this study were in accordance with and approved by the East Tennessee State University Animal Care and Use Committee and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Drug dosage

Animals were administered of 10 mg/kg of quinpirole based on findings by Kostrzewa et al. (1990) that this dose is sufficient to produce long-term priming of the dopamine D2 receptor. Nicotine bitartrate (Sigma-Aldrich, St. Louis, MO) was diluted in saline to 0.5 mg/kg free base (pH ~ 7.0). For Experiment 1, S(-)-eticlopride hydrochloride (Sigma-Aldrich, St. Louis, MO) was diluted in saline to 0.1 mg/kg based on findings by Palmatier and Bevins (2002) that this dose is sufficient to impair locomotor activity in a nicotine conditioned hyperactivity paradigm. For Experiment 2, L-nafadotride (Sigma-Aldrich, St. Louis, MO) was diluted in saline to 0.1 mg/kg based on findings by Murray and Bevins (2007) that this dose maintains specificity for the dopamine D3 receptor subtype.

Drug Treatment

Neonatal. On postnatal days 1 to 21 (P1-P21), animals were given a single daily intraperitoneal (i.p.) injection of either quinpirole (10 mg/kg) or saline. Animals were randomly assigned to treatment group in order to maintain relative sameness in group size. All animals gained weight normally during drug treatment.

Adolescent. Animals were randomly assigned to treatment groups on P28. Animals were drawn from 9 litters and were assigned to the following treatment group with the first drug representing neonatal treatment, the second representing the administered antagonist (or Saline if control group), and the third representing adolescent treatment: Saline-Saline-Saline (SSS), Saline-Saline-Nicotine (SSN), Quinpirole-Saline-Saline (QSS), Quinpirole-Saline-Nicotine (QSN) Saline-Eticlopride-Saline (SES); Saline-Eticlopride-Nicotine (SEN) Quinpirole-Eticlopride-Saline (QES), Quinpirole-Eticlopride-Nicotine (QEN), Saline-Nafadotride-Saline (SNS), Quinpirole-Nafadotride-Saline (QNS), Saline-Nafadotride-Nicotine (SNN), or Quinpirole-Nafadotride-Nicotine (QNN). Animals in the SS, QS, SES, QES, SNS, and QNS treatment groups served as the unpaired group (Bevins & Palmatier, 2003) which served as controls for the context-nicotine paired groups. The rationale for this group is that these animals received the same number of exposures to nicotine but did not have the drug paired within an environmental context.

Apparatus

The two locomotor arenas were Plexiglas boxes painted black and measuring 72 cm on all sides. The identical locomotor arenas served as the conditioning contexts. According to classical conditioning, the locomotor arenas serve as the conditioned stimulus that elicits the conditioned response of horizontal locomotor activity after being reliably and repeatedly paired

with the unconditioned stimulus, nicotine. All activity was monitored by an automated behavioral scanning system (Any Maze, Stoelting Co., Wood Dale, IL). A grid was superimposed on the floor of the locomotor arena using the Any-Maze scanning system. Number of lines crossed served as the dependent variable of horizontal activity. Two locomotor arenas were used because the behavioral scanning system allowed for simultaneous data collection of two subjects.

Behavioral testing procedure

Beginning on P29, animals were habituated to the locomotor arena for 3 consecutive days. On each day of habituation, all animals were given an injection of saline and placed into the locomotor arena 10 minutes later. Each habituation session lasted for 10 minutes. This was done to allow animals to adapt to the locomotor apparatus and to the injection procedure. Beginning on P32, animals were injected i.p. with either nicotine (0.5 mg/kg free base) or saline. Animals were injected i.p. with either eticlopride (0.1 mg/kg), nafadotride (0.1 mg/kg), or saline 10 minutes before the nicotine or saline injection. Ten minutes after the adolescent treatment injection, the animals were placed into the locomotor arena for a 10-minute session. This procedure was repeated every other day for 18 days resulting in 9 days of sensitization testing.

Animals in the unpaired groups (SSS, QSS, SES, QES, SNS, and QNS) were administered saline before being placed into the locomotor arena and nicotine in the home cages 2 h after conditioning to control for nicotine exposure. The unpaired groups are so named because they receive the same number of exposures to nicotine; however, the drug is not repeatedly or reliably paired with the conditioned context. For the conditioned hyperactivity test,

all animals were given a saline injection and 10 minutes later were placed into the locomotor arena for one 10-minute session and behavior was recorded.

Research Design and Data Analysis

An initial 2 (sex) x 2 (neonatal drug treatment) x 2 (antagonist) x 2 (adolescent) x 5 (repeated measure) five-way mixed factorial ANOVA was performed for both the eticlopride and nafadotride treated groups. There was a significant sex main effect in each experiment. Therefore, sex effects was analyzed separately using two 2 (neonatal) x 2 (antagonist) x 2 (adolescent) x 9 (day of testing) ANOVAs. The Newman-Keuls post hoc test was used to analyze significant interactions.

Planned comparisons were used to analyze conditioned hyperactivity data collected during the drug-free trial. These comparisons were made to answer the following specific questions. Group SSS was compared to group SSN to determine whether nicotine elicited conditioned-hyperactivity. Group SSN was compared to group SSN to determine the effect, if any, of D2-priming on conditioned-hyperactivity. Group SEN was compared to group SSN to examine the effect of eticlopride on conditioned hyperactivity. Finally, Group QEN was compared group QSN to examine the effect of eticlopride on conditioned-hyperactivity in D2-primed rats. The four planned group comparisons for conditioned hyperactivity were the same for both males and female rats.

CHAPTER 3

RESULTS

Sex Differences in Nicotine Sensitization for Rats Administered Eticlopride

Initial analyses. Although there were several significant main effects and interactions, the initial analysis focused on analyzing sex differences and their interaction with drug treatment. The initial 2 x 2 x 2 x 2 x 9-way ANOVA of sex, neonatal, antagonist (eticlopride or saline), adolescent, and day of testing yielded a significant main effect of sex $F(1,94) = 10.11, p < .002$, significant two-way interactions of Sex x Antagonist $F(1,94) = 3.68, p < .05$, Sex x Day of Testing $F(8,752) = 2.01, p < .04$, a significant three-way interaction of Sex x Neonatal Drug Treatment x Day of Testing $F(8,752) = 2.30, p < .04$ and Sex x Antagonist x Day of Testing $F(8,752) = 1.98, p < .04$.

Analysis of the significant sex main effect revealed that overall females were significantly more active than males. Post hoc analysis of the significant interactions with the Newman-Keuls test revealed that females administered saline in the absence of an antagonist demonstrated significantly higher levels of activity than males administered saline, but females and males were equivalent in their horizontal activity when administered eticlopride. Females demonstrated higher levels of horizontal activity as compared to males on days 1-3 and 7-8. In particular, females neonatally administered saline demonstrated higher levels of horizontal activity as compared to males on days 4 and 8, and females administered saline demonstrated significantly higher levels of horizontal activity as compared to males administered saline or eticlopride on days 3-5 and 7-9. In summary, adolescent females demonstrated significantly higher levels of horizontal activity than adolescent males, an effect that has been repeatedly

observed in the research literature (Becker, 1999; Booze et al., 1999; Elliot et al., 2005; Faraday et al., 2003; Harrod et al., 2004; Kanyt et al., 1999; Perna, 2006).

Horizontal Activity for Male Rats Administered Eticlopride. A 2 x 2 x 2 x 9 four-way repeated measures ANOVA revealed a significant main effect of Antagonist $F(1,47) = 50.99$, $p < .001$, significant two-way interactions of Antagonist x Adolescent Drug Treatment $F(1,47) = 5.25$, $p < .027$, Neonatal Drug Treatment x Day of Testing $F(8,376) = 2.67$, $p < .007$, Antagonist x Day of Testing $F(8,376) = 15.80$, $p < .001$, Adolescent Drug Treatment x Day of Testing $F(8,376) = 7.72$, $p < .001$, and significant three-way interactions of Neonatal Drug Treatment x Antagonist x Day of Testing $F(8,376) = 2.12$, $p < .033$, and Adolescent Drug Treatment x Antagonist x Day of Testing $F(8,376) = 3.56$, $p < .001$. Finally, a significant four-way interaction of Neonatal Drug Treatment x Antagonist x Adolescent Drug Treatment x Day of Testing $F(8,376) = 2.94$, $p < .02$. Most importantly, this interaction revealed that Male Group QSN demonstrated significantly higher levels of horizontal activity than all other groups at days 7 through 9. Also, Male Group SSN demonstrated significantly higher levels of horizontal activity than Group SSS at days 7 through 9 demonstrating sensitization in this group. Results of this analysis are shown in Figure 1.

Figure 1. Horizontal Activity for Males Administered Eticlopride

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

* indicates significance greater than control group SSS ($p < .05$)

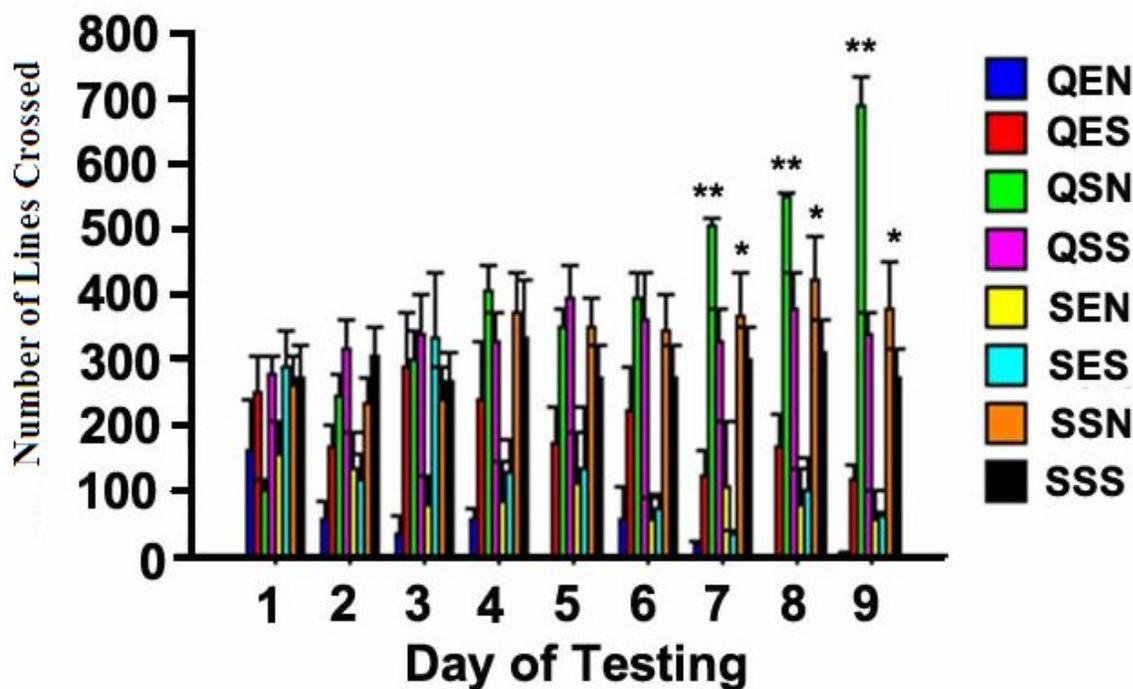


Figure 1. Horizontal Activity for Males Administered Eticlopride

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

* indicates significance greater than control group SSS ($p < .05$)

Horizontal Activity for Female Rats Administered Eticlopride. A $2 \times 2 \times 2 \times 9$ four-way ANOVA revealed significant main effect of Antagonist $F(1,49) = 126.67, p < .001$, significant two-way interactions of Antagonist x Adolescent Drug Treatment $F(1,49) = 7.89, p < .007$, Neonatal Drug Treatment x Day of Testing $F(8,392) = 2.73, p < .006$, and Antagonist x Day of Testing $F(8,392) = 9.51, p < .001$, two significant three-way interactions of Antagonist x Adolescent Drug Treatment x Day of Testing $F(8,392) = 3.69, p < .001$, and Neonatal Drug Treatment x Adolescent Drug Treatment x Day of Testing $F(8,392) = 2.14, p < .03$. Results of this analysis are shown in Figure 2.

First, the significant main effect of Antagonist demonstrated that eticlopride resulted in lower levels of horizontal activity compared to all other groups. Post hoc analysis of the two-way interactions revealed that animals administered nicotine or saline in adolescence demonstrated significantly higher levels of horizontal activity compared to animals administered eticlopride or saline. Females neonatally treated with saline demonstrated significantly higher levels of horizontal activity at days 4 and 8. Females administered eticlopride demonstrated significantly lower levels of horizontal activity from days 2 through 9.

Analysis of the three-way interaction of Antagonist x Adolescent Drug Treatment x Day of Testing revealed that eticlopride effectively produced a significant decrease of horizontal activity in females administered nicotine in adolescence as compared to animals administered eticlopride plus saline during at days 3 and 6 through 9. Females neonatally administered saline and given saline, as opposed to an antagonist, and nicotine in adolescence (Group SSN) had significantly higher levels of horizontal activity at days 6 through 8. Interestingly, Group SSN activity levels drop to control levels on day 9.

Finally, and most importantly, analysis of the significant three-way interaction of Neonatal Drug Treatment x Adolescent Drug Treatment x Day of Testing revealed that females administered saline neonatally and nicotine in adolescence demonstrated significantly higher levels of horizontal activity at days 5 through 8 than all other groups. Additionally, females administered quinpirole neonatally and nicotine in adolescence (Group QSN) were equivalent to the control group of females administered saline neonatally during antagonism and in adolescence (Group SSS). This effect replicates past work in adolescence that has shown that D2-primed females administered nicotine appear to decrease horizontal activity due to increases

in stereotypic behaviors, such as grooming, paw treading, and vacuous chewing movements late in nicotine sensitization testing (Perna, 2006).

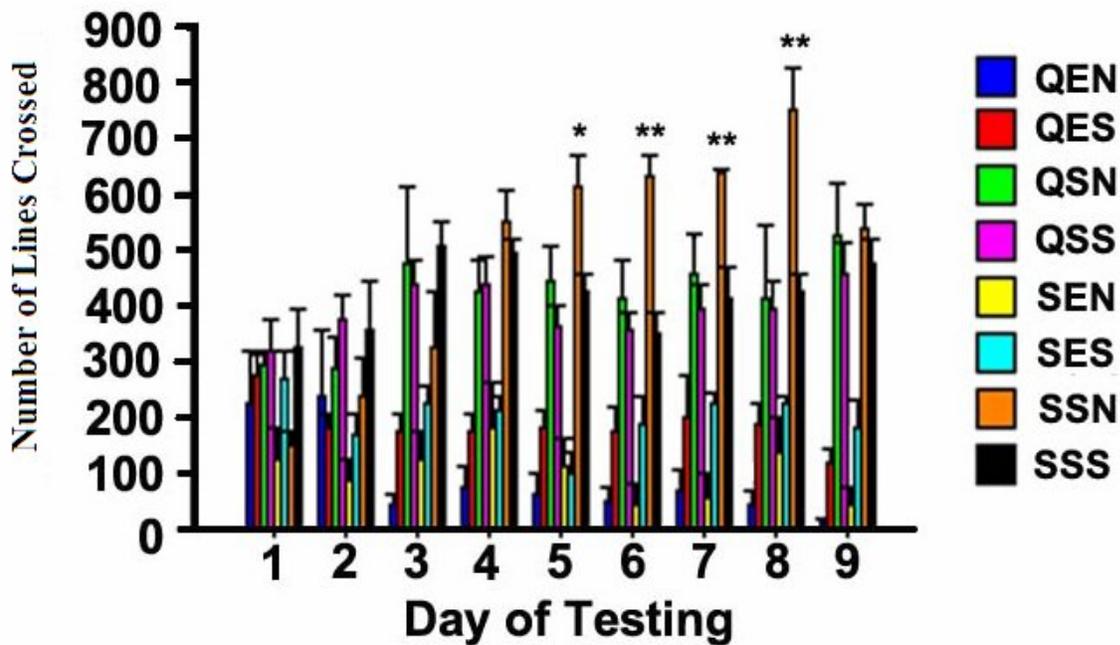


Figure 2. Horizontal Activity for Females Administered Eticlopride

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

* indicates significance greater than control group SSS ($p < .05$)

Sex Differences in Nicotine-Conditioned Hyperactivity for Rats Administered Eticlopride

Conditioned Hyperactivity for Male Rats Administered Eticlopride. There were four planned group comparisons performed for conditioned hyperactivity in males: Groups SSN to SSS, SSN to QSN, SEN to SSN, QEN to QSN, and SEN to SSN (see Figure 3). For the planned group comparison of Group SSN to SSS, the independent t-test was not significant $t(12) = .41$, $p = .69$, demonstrating that nicotine did not produce conditioned hyperactivity in adolescent males. However, the planned comparison of Group SSN to QSN, was significant $t(12) = 2.63$, $p < .022$, and Group QSN demonstrated a significant increase in horizontal activity as compared

to Group SSN. The planned comparisons of SEN to SSS $t(11) = .65, p=.53$ and QEN to QSN were also not significant $t(12) = .92, p=.38$.

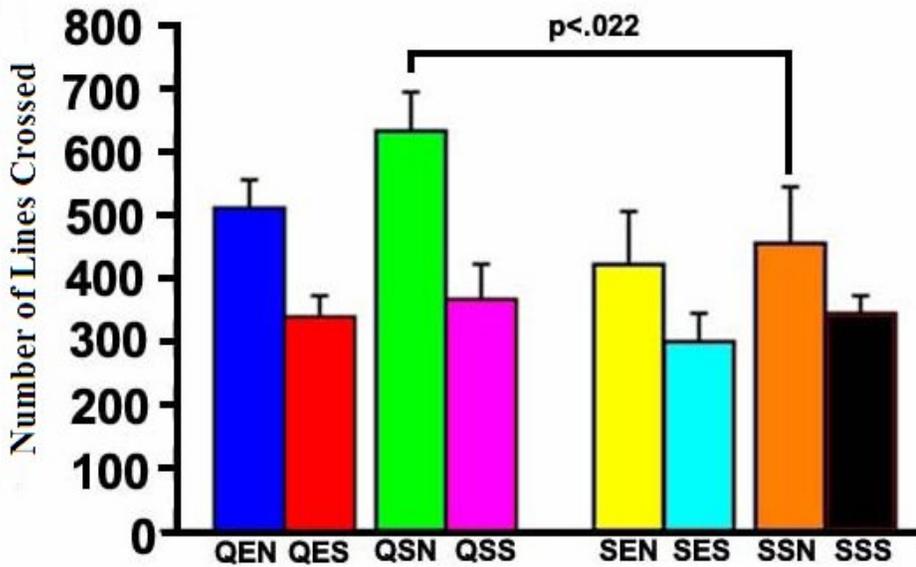


Figure 3. Conditioned Hyperactivity for Males Administered Eticlopride

Conditioned Hyperactivity for Female Rats in Administered Eticlopride. The same four planned group comparisons performed for males were also performed to examine conditioned hyperactivity in females: Groups SSN to SSS, SSN to QSN, SEN to SSN, and QEN to QSN (see Figure 4). For the planned group comparison of Group SSN to SSS, the independent t-test was significant $t(10) = 3.54, p<.006$. Group SSN demonstrated a significant higher level of activity on the drug free test than Group SSS. Likewise, Group SSN demonstrated significantly higher levels of activity on the drug free test than Group QSN $t(9) = 2.59, p<.03$. The planned comparison of Groups QEN and QSN was not statistically significant $t(12) = .27, p=.78$. The planned comparisons of Group SSN to SEN revealed another significant effect $t(12) = 2.27, p<.04$.

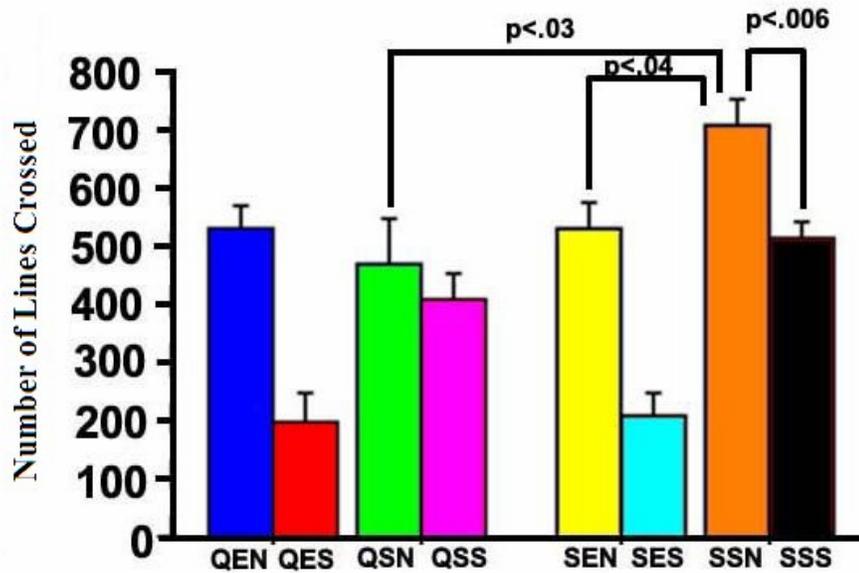


Figure 4: Conditioned Hyperactivity for Females Administered Eticlopride

Sex Differences in Nicotine Sensitization for Rats Administered Nafadotride.

Initial Analyses. Similar to the analysis of rats administered eticlopride, there were several significant main effects and interactions, but in this initial analysis we were primarily interested in analyzing sex differences and their interaction with drug treatment. An initial nine-way ANOVA with sex, neonatal, antagonist, adolescent, and day of testing ($2 \times 2 \times 2 \times 2 \times 9$) yielded a significant main effect of sex $F(1,77) = 7.43, p < .008$, significant two-way interactions of Sex x Antagonist $F(1,94) = 3.68, p < .05$, Sex x Day of Testing $F(8,616) = 3.22, p < .001$. Two significant four-way interactions of Sex x Neonatal Drug Treatment x Antagonist x Day of Testing $F(8,616) = 3.0, p < .002$ and Sex x Antagonist x Adolescent Drug Treatment x Day of Testing.

The analyses of the first of these two higher order interactions revealed increased horizontal activity in females administered saline as opposed to the D3 antagonist nafadotride at days 4, 5, 8, and 9. There were no significant group differences in males. The analysis of the latter of these two higher order interactions revealed that females neonatally treated with saline

and administered nicotine during adolescent treatment demonstrated significantly higher levels of horizontal activity than all other groups at days 5 through 9, and this effect was blocked by nafadotride. Males neonatally administered saline and given nicotine in adolescence also demonstrated a significant increase in horizontal activity at days 6, 8, and 9 as compared males given saline at both time points. Again, this effect was blocked by nafadotride. Based on these sex differences, males and females were analyzed separately.

Horizontal Activity for Male Rats Administered Nafadotride. A 2 x 2 x 2 x 9 four-way ANOVA revealed no significant main effects. There significant two-way interactions of Antagonist x Adolescent Drug Treatment $F(1,45) = 7.37, p < .009$, Antagonist x Day of Testing $F(8,360) = 3.19, p < .002$, and Adolescent Drug Treatment x Day of Testing $F(8,360) = 8.06, p < .001$. There was a significant three-way interaction of Neonatal Drug Treatment x Antagonist x Day of Testing $F(8,360) = 3.50, p < .001$. Finally, there was a significant four-way interaction of Neonatal Drug Treatment x Antagonist x Adolescent Drug Treatment x Day of Testing $F(8,360) = 2.01, p < .04$. Results of this analysis are shown in Figure 5.

Post hoc analysis of the most complex interaction revealed that Group QSN had significantly higher levels of horizontal activity on Days 7 through 9 compared to all other groups. Group QNN demonstrated a significant increase in horizontal activity compared to Group QSN on day 1 but was equivalent to controls, Group SSS, throughout testing. This result demonstrates that nafadotride blocked locomotor sensitization to nicotine in D2-primed males and also blocked the initial hypoactive response to nicotine. However, in non D2-primed males nafadotride blocked locomotor sensitization to nicotine but, interestingly, produced an initial hypoactive response in Group SNN as compared to Group SSN at day 1.

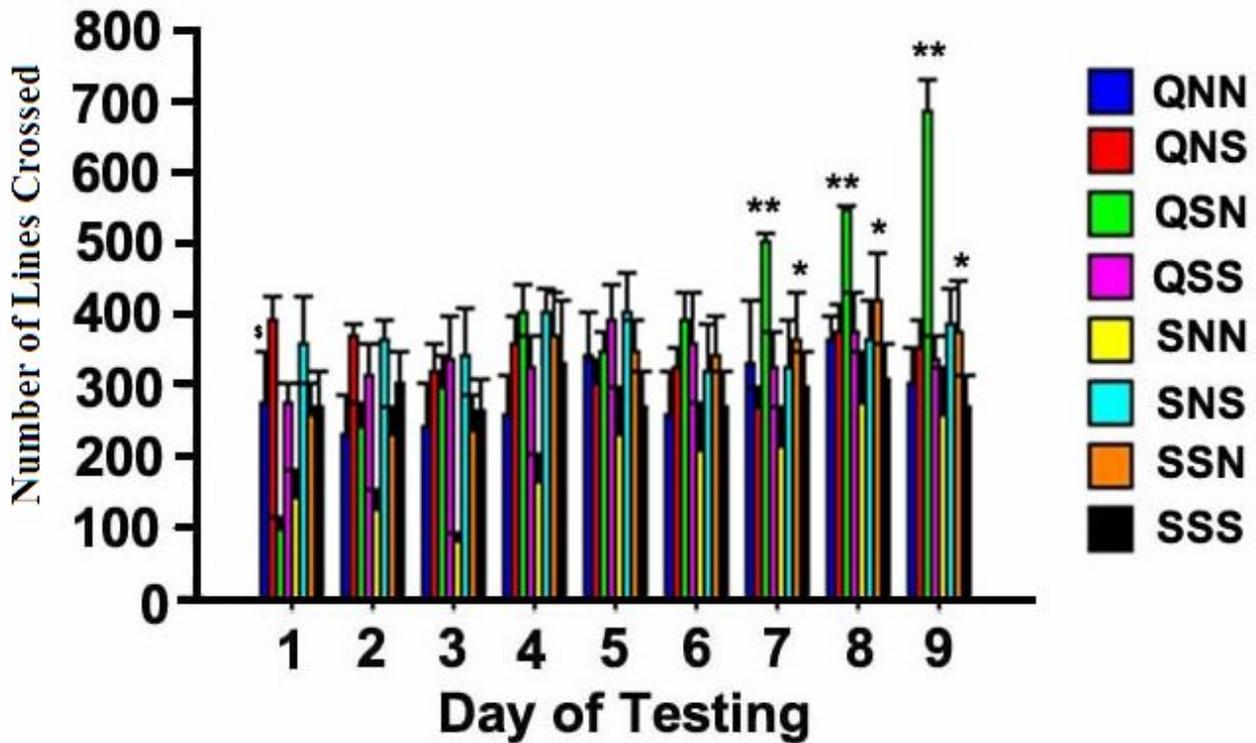


Figure 5. Horizontal Activity for Males Administered Nafadotride

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

* indicates significance greater than control group SSS ($p < .05$)

\$ indicates Group QNN was significantly greater than Group QSN on Day 1

Horizontal Activity for Female Rats Administered Nafadotride. A $2 \times 2 \times 2 \times 9$ four-way ANOVA revealed a significant main effect of Antagonist $F(1,39) = 7.94, p < .008$, a significant two-way interaction of Adolescent Drug Treatment \times Day of Testing $F(8,312) = 7.66, p < .001$, a significant three-way interactions of Antagonist \times Adolescent Drug Treatment \times Day of Testing $F(8,312) = 2.93, p < .003$, and a significant four-way interaction of Neonatal Drug Treatment \times Antagonist \times Adolescent Drug Treatment \times Day of Testing $F(8,312) = 2.14, p < .04$. These results are shown in Figure 6.

Most importantly, analysis of this four-way interaction revealed that Group SSN demonstrated significantly higher levels of horizontal activity at days 5 through 8. Group QNN

activity levels were equivalent to controls, Group SSS, at days 2 through 9. Groups QNN and SNN, nafadotride groups that received nicotine, demonstrated significant hypoactivity on day 1 compared to control Group SSS. Interestingly, nafadotride blocked sensitization to nicotine; it did not block the initial hypoactive response to nicotine on day 1. Female Group QSN demonstrated significantly lower levels of horizontal activity as compared to Group SSN at days 5 through 8, presumably due to increased stereotypy in this group.

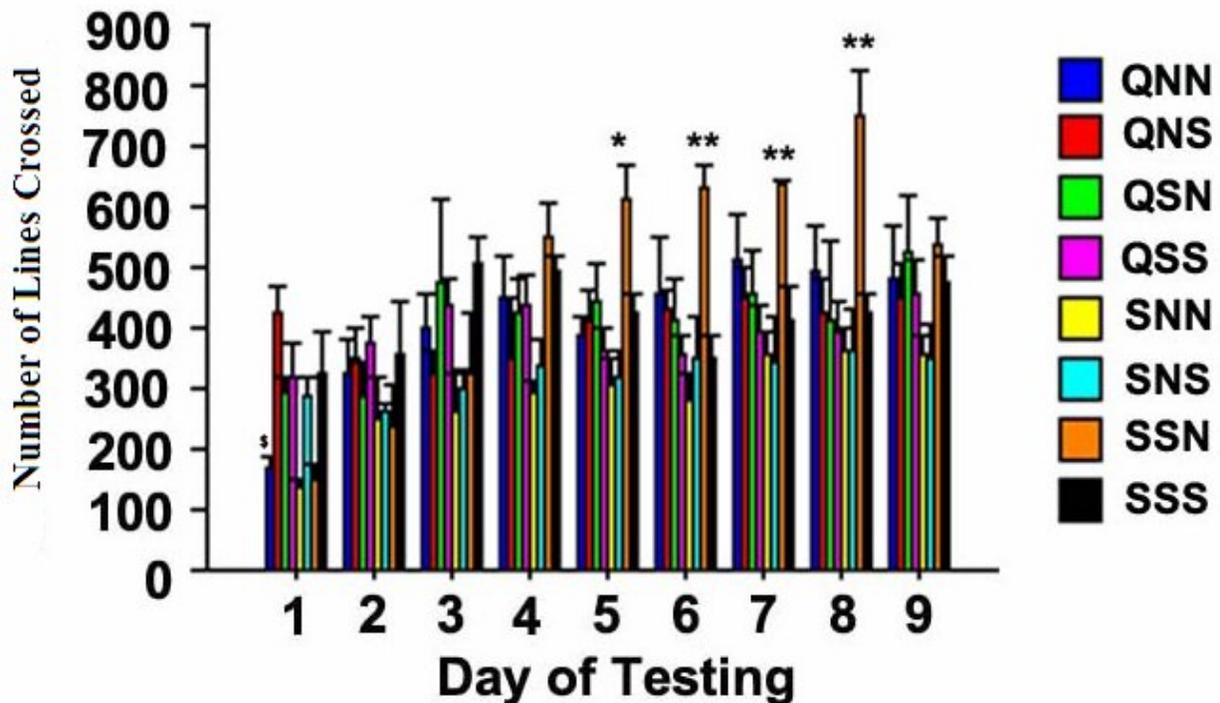


Figure 6. Horizontal Activity for Females Administered Nafadotride

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

* indicates significance greater than control group SSS ($p < .05$)

\$ indicates Group QNN demonstrated significantly lower activity levels as compared to Group SSS on Day 1

Sex Differences in Nicotine-Conditioned Hyperactivity for Rats Administered Nafadotride

Conditioned-Hyperactivity for Male Rats Administered Nafadotride. Only two planned comparisons were performed on conditioned hyperactivity: The comparison of Groups SNN to SSN was made to examine if nafadotride blocked conditioned hyperactivity. The comparison of Group QNN to QSN was performed to examine the effect of nafadotride on nicotine-conditioned hyperactivity in D2-primed rats. The comparison of Groups SNN to SSN was not statistically significant $t(8) = .044, p=.96$. In the comparison of QNN to QSN, the t-test was statistically significant $t(10) = 2.97, p<.01$. Group QSN demonstrated significantly higher levels of horizontal activity compared to Group QNN, demonstrating that nafadotride blocked nicotine conditioned hyperactivity in adolescent males. These results are shown in Figure 7.

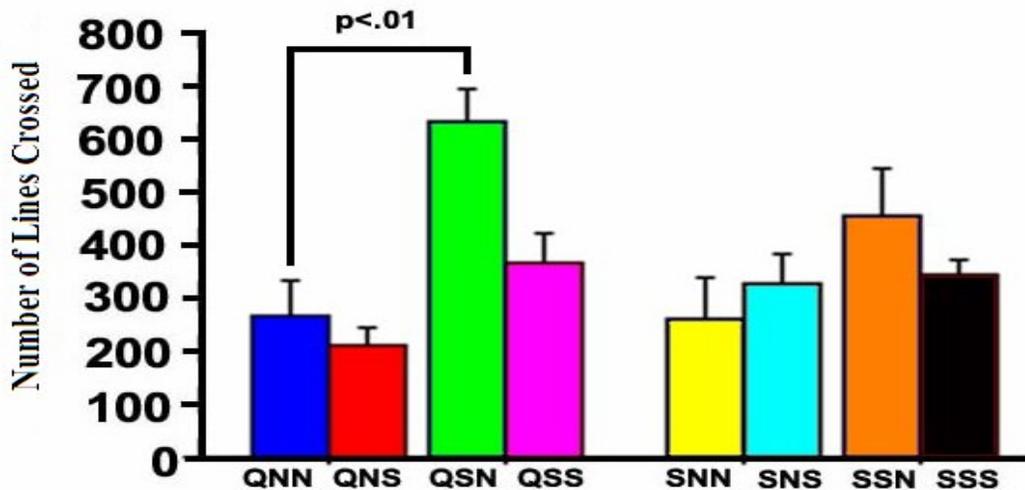


Figure 7. Conditioned Hyperactivity for Males Administered Nafadotride

Conditioned-Hyperactivity for Female Rats Administered Nafadotride. Similar to males, only two planned comparisons were performed on conditioned hyperactivity. The first comparison of QNN to QSN was designed to test whether D2-priming effects on nicotine conditioned increases in locomotor activity were blocked by nafadotride. The second comparison

of Groups SNN to SSN was designed to test whether nafadotride blocked nicotine conditioned increases in locomotor activity in non D2-primed female adolescent rats. The planned comparison of Group QNN to QSN was not significant $t(12) = 1.34, p=.21$. However, the planned comparison of Groups SNN to SSN was significant $t(9) = 3.09, p<.013$. The results of this analysis are shown in Figure 8.

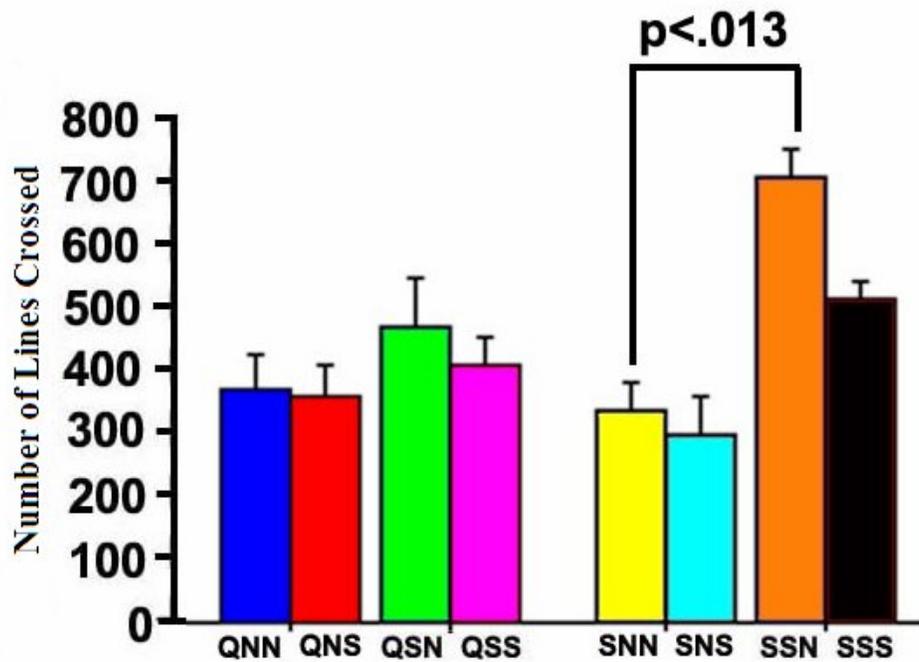


Figure 8. Conditioned Hyperactivity for Females Administered Nafadotride

CHAPTER 4

DISCUSSION

The results of the present study support previous findings that priming of the dopamine D2 receptor results in enhanced behavioral sensitization to nicotine (Perna, 2006). In addition, this study also demonstrated that antagonizing the D2 and D3 receptor subtype blocks this sensitization suggesting differential roles of dopamine receptor subtypes in the induction of nicotine sensitization. Antagonism of the D2 receptor blocked nicotine-conditioned hyperactivity in female rats but not male rats. Nafadotride blocked nicotine-conditioned hyperactivity in D2-primed male rats but not female rats. These results suggest sex-based differential roles of the D2 and D3 receptor subtypes on the association of nicotine with conditioned contextual cues.

Nicotine Sensitization

D2 priming blocked the initial hypoactive behavioral response typically observed after administration of nicotine (Brown & Kolb, 2001). This result is in agreement with previous findings that adolescent rats also showed less sensitivity to nicotine's initial hypoactivity effects (Elliot et al., 2005). Overall, D2 primed rats that received nicotine demonstrated a significant increase in activity compared to other groups and reached the asymptote of locomotor response to nicotine more rapidly as compared to controls. Results showed that in male rats D2 priming enhanced sensitization to nicotine as compared to non D2-primed rats. In female D2-primed rats administered nicotine demonstrated an increased hypoactive response after initial sensitization. The quantitative decrease in horizontal activity after initial sensitization is hypothesized to result from qualitatively observed increases in stereotypic behaviors such as sniffing, licking, grooming, and rearing movements (Kelley, 2001; Lepekhina, & Tsitsurina, 2007). These observations are in agreement with previous findings that female rats show increased periods of

stereotypic behavior after administration of the psychostimulants amphetamine (Milesi-Halle, McMillan, Laurenzana, Byrnes-Blake, & Ownes, 2007) and nicotine (Perna, 2006).

Another factor that may lead to increased stereotypic behavior in female rats is increased dopamine D1 receptor sensitivity as compared to male rats (Schindler & Carmona, 2002). Vezina and Stewart (1989) posit that the D1 receptor subtype plays a more important role in locomotor sensitization to psychostimulants as compared to the D2 receptor subtype. However, knockout studies have shown that D2 receptor mutants demonstrate more drastic reductions in motor function as compared to D1 receptor mutants (Kobayashi et al., 2004). Reductions in locomotor activity in D1 mutants are dependent upon the experimental protocol used suggesting an important interrelationship between receptor subtypes in the control of motor activity (Kobayashi et al.). However, differential findings of the importance of the D1 versus D2 receptor in locomotor sensitization may reflect species specific differences in receptors.

Nicotine induced dopamine release in the nucleus accumbens, striatum, and to some extent the frontal cortex, is modulated by presynaptic nicotinic acetylcholinergic receptors (nAChRs) on dopaminergic terminals of neurons in the nucleus accumbens (Grady, Marks, Wonnacott, & Collins, 1992; Marshall, Redfern, & Wonnacott, 1997). Nicotine also blocks the inhibitory action of dopamine at the presynaptic D2 autoreceptor resulting in additional synaptic availability of dopamine (Barik & Wonnacott, 2006). Increased receptor sensitivity combined with increased neurotransmitter availability may, in part, account for the augmented behavioral sensitization and sex differences in behavior observed in the present study.

Antagonism of Dopamine Receptor Subtypes

D2 Receptor. In agreement with Bevins and Palmatier (2002), eticlopride blocked induction of nicotine sensitization in male and female rats. Interestingly, eticlopride was more effective at blocking sensitization to nicotine in D2-primed as compared to non D2-primed rats which is presumably due to increased sensitivity of the D2 receptor as a result of neonatal receptor priming (Kostrzewa, 1995), and eticlopride is binding more effectively to primed D2 receptors.

D3 Receptor. Inconsistent with findings by Murray and Bevins (2007), the D3 antagonist nafadotride blocked sensitization in both D₂-primed and non D₂-primed male and female rats. This result is also inconsistent with previous findings that nafadotride failed to block expression of sensitization to the psychostimulant cocaine (Betancur et al., 2001; Filip, Papla, & Czepeil, 2002) but is consistent with past work with amphetamine (Chiang, Chen, & Chen, 2003; Richtand et al., 2000). This may be a spurious result, but it may also be that the present results reflect age differences in dopamine D3 receptor function, as all of these past studies have used adults as subjects.

Adolescent D3 receptor levels have been shown to be at only 40% of adult levels during the adolescent period (Faraday et al. 2003; Shram et al., 2006). Thus, it may be that these receptors increase sensitivity due to their low number in the brain and play a larger role in locomotor sensitization to nicotine in adolescence than in adulthood. On the other hand, it may be that adolescent animals are more likely to demonstrate increases in stereotypic behaviors, as observed in the present study in nicotine given to D2-primed females. Thus horizontal activity may not be the most accurate behavioral measure for activation to psychostimulant drugs. One important consistency however is that nafadotride fails to block sensitization to amphetamine

(Chiang et al., 2003). Both amphetamine (Nowak, Brus, & Kostrzewa, 2001) and nicotine (Benowitz, 2008) produce an increase in dopamine release and block the presynaptic dopamine D2 receptor autoreceptor (Geldwert et al., 2006). Nicotine and amphetamine may have similar actions at the synapse and these actions may be blocked by D3 antagonism. Further studies are needed to examine sex differences in D3 receptor density and the possible effects of density on the associative conditioning properties of nicotine.

Dopamine Receptor Density in Adolescence. Andersen and Teicher (2000) have shown sex differences in dopamine receptor density over the periadolescent period, which has been defined as occurring from approximately P28 to P60 (Smith, 2003), as compared to the adult period. Dopamine D2 receptor density in the striatum of male rats increases 1.4 fold between P25 and P40, whereas D2 receptor density in the striatum of female rats is increased by only 31% during this time. Male D2 receptor density sharply decreases by 55% by adulthood, whereas females show little overproduction during the same developmental period. Interestingly, during adulthood, there were no sex differences in the density of D1 and D2 receptors (Andersen & Teicher).

The results of this study support previous findings that adolescent rats, particularly D2-primed adolescents, demonstrated a more robust sensitization to nicotine as compared to adult rats (Elliot et al., 2005; Perna, 2006), based on the increases in dopamine receptor density during the adolescent period. Further, these increases in dopamine receptor density suggest that drugs enhancing the dopaminergic system, such as addictive drugs, may have more prevalent effects during this period. Certainly, the results shown here support this effect in males, as D2-primed males demonstrated an enhanced sensitization to nicotine, suggesting increased behavioral activation to nicotine.

Alternative Mechanisms. Several studies have shown that multiple neurotransmitter systems are affected by nicotine including the cholinergic (Brown et al, 2006; Quarta et al., 2007), glutamatergic (Balfour et al., 1996; Birrell & Balfour, 1998; Domino, 2001; Shoaib et al., 1994; Suemaru et al., 1993), and GABAergic systems (Carlsson, 2000). Blockade of nicotinic or glutamatergic receptors has been shown to block sensitization to nicotine, suggesting that the cholinergic or glutamatergic systems may also play a role in sensitization to nicotine.

Nicotinic receptors are located both pre- and postsynaptically, and the presynaptic nicotinic receptor has been shown to be located on cholinergic, glutamatergic, serotonergic, as well as dopaminergic neurons (McKay, Placzek, & Dani, 2007). This ubiquity of the nicotinic receptor throughout the brain makes the action of nicotine difficult to understand. Certainly, had an antagonist to another neurotransmitter system been used in the present study, it very well may have blocked sensitization to nicotine, and it is realized that the mechanism underlying the behavioral results observed in the present study may be mediated by several neurotransmitter systems. Regardless, current evidence still supports increased dopamine release in the nucleus accumbens via the direct agonist actions of nicotinic receptors located in the VTA (Di Chiara et al., 2004; Perna, 2006; Shim et al., 2001; Shoaib et al., 2004).

Nicotine-Conditioned Hyperactivity

Results suggest that D2 priming enhanced conditioned hyperactivity to nicotine in males, and this effect was not blocked by the D2 antagonist eticlopride. Further, non D2-primed males administered nicotine did not demonstrate conditioned hyperactivity. Results showed that eticlopride, a dopamine D2 antagonist, blocked conditioned hyperactivity in females but not in males, suggesting that the D2 receptor plays a role in the association of nicotine with a conditioned context, at least in females. This is somewhat consistent with past research.

Schindler and Carmona (2002) have shown that adult female rats demonstrate an increase in locomotor activation to acute quinpirole. The result that females administered eticlopride failed to demonstrate nicotine conditioned hyperactivity further supports results showing there are sex differences in D2 receptor function, especially in adolescence.

The results using the dopamine D3 antagonist nafadotride were more complex. First, nafadotride blocked conditioned hyperactivity in non D2-primed females. This result, taken with the findings with the D2 antagonist eticlopride, suggests that both the D2 and D3 receptor play a role in the conditioned effects of nicotine in females. Further, because D2-primed females did not demonstrate conditioned hyperactivity to nicotine, it does not appear that priming of the D2 receptor in females affects associative effects of nicotine as it relates to behavioral activation.

Nafadotride was effective in blocking conditioned hyperactivity in D2-primed males, but because non D2-primed males did not demonstrate nicotine conditioned hyperactivity, it did not produce any effects in this group. Thus, the D3 receptor may play a more important role in expression of behavioral activation to nicotine in males as compared to females; however, there is a reasonable alternative explanation.

To produce priming of the dopamine D2 receptor, ontogenetic quinpirole was administered. Quinpirole is a dopamine D2/D3 agonist, thus, both D2 *and* D3 receptors may be primed. Further, work by Collins et al. (2005) has shown that the D3 receptor is more important in yawning behavior as compared to the D2. All of this taken together suggests that the D3 receptor may also be primed in these animals and may be playing a more important role in nicotine association in males as compared to the D2 receptor. Increased sensitivity of the D3 receptor, which has been shown to play an important role in the association of nicotine with a conditioned context (Le Foll et al., 2005), may have resulted in increased behavioral response to

the conditioned context during the drug-free trial. This is especially interesting considering the importance of the dopamine system in addiction to nicotine, and sex differences that have been shown in not only schizophrenics (Leung & Chue, 2000) but in the normal population (Bigos et al., 2008).

Initially, the finding that non-D2-primed females exhibited a significantly higher conditioned hyperactivity response as compared to D2-primed females and controls is surprising. However, previous studies by Harrod et al. (2004) have shown decreased numbers of D3 receptors in the NAcc in female rats as compared to males after repeated intravenous nicotine administration. The reduction in supersensitized D3 receptors in the accumbens shell of D2-primed female rats as a result of chronic nicotine administration may partially account for this finding.

The importance of the D3 receptor in association of drugs of abuse with contextual stimuli may be due to the increased number of receptors in the accumbens shell as compared to the core (Stanwood, McElligot, Lu, & McGonigle, 1997). As previously mentioned, the shell of the nucleus accumbens (AccSh) has been proposed as a histological extension of the amygdala, the emotional center of the brain that is thought to play an important role in associative learning in rewarding situations (Everitt, Morris, O'Brien, & Robbins, 1991; Meredith, Callen, & Scheuer, 2002). Therefore, it is not surprising that administration of the D3 antagonist more effectively blocked nicotine-conditioned hyperactivity as compared to the D2 antagonist eticlopride.

Clinical Relevance

The findings of the current studies present several considerations for the treatment of nicotine addiction, particularly in the schizophrenic population. The apparent importance of the D2 receptor in nicotine sensitization suggests that treatments targeting the D2 receptor may be useful for simultaneous cessation of smoking and treatment of the positive symptoms of schizophrenia as it appears. The increased sensitivity of D2-primed rats to nicotine exposure provides additional support for D2-priming as an animal model of schizophrenia. This result is consistent with human schizophrenia literature focused on nicotine addiction in this group (Salin-Pascual, Alcocer-Castillejos, & Alejo-Galarza, 2003). Bigos et al. (2008) have recently shown that smoking impacts the metabolism of and may therefore reduce the effectiveness of olanzapine in the treatment of schizophrenia. Therefore, drug interactions must always be considered when developing new treatments.

As noted by Le Foll et al. (2007), the D3 receptor represents another potential target for the treatment of nicotine addiction. Findings from this study support the role of D3 receptors in the association of nicotine to conditioned stimuli as evidenced by the blockade of nicotine-conditioned hyperactivity during the drug-free trial. Targeting the D3 receptor may be especially beneficial for male schizophrenic patients indicated by the result that D2-primed male rats showed the highest levels of conditioned hyperactivity. The D3 receptor provides a potential target for treatment of cravings associated with stimuli associated with smoking. Perhaps development of a pharmacological agent that antagonizes both the D2 and D3 receptor will aid in the reduction of nicotine addiction by relieving both the physical and associative addiction to nicotine that smokers suffer.

Conclusion

The aim of this investigation was to determine the effect of a nicotine-conditioned context on locomotor hyperactivity in an animal model of D2-priming, and whether conditioned hyperactivity could be blocked by the D2 antagonist eticlopride or the D3 antagonist nafadotride. The results of this study demonstrate that the D2 and D3 dopamine receptor subtypes are important in both the locomotor activating effects of nicotine and also the association of nicotine with conditioned stimuli, particularly environmental cues. More specifically, results demonstrated that a nicotine-conditioned context can elicit hyperactivity and that hyperactivity is blocked by both D2 and D3 receptor antagonism in female rats and D3 receptor antagonism in male rats. These results also demonstrate differences in locomotor activity levels of D2-primed and non D2-primed rats suggesting that nicotine will also have different effects in schizophrenic patients as compared to the general population.

These results support previous findings that demonstrate the existence of sex differences in locomotor sensitization and hyperactivity that may result from differences in receptor sensitivity or density (Harrod et al., 2004; Le Foll, Diaz, et al., 2003; Schindler & Carmona, 2002) during different developmental periods. Sex differences in receptor subtype density during adolescence, receptor sensitivity throughout development, and the introduction of differential hormone levels at sexual maturity are all factors that impact nicotine's effects on behavior. This study highlights the need for additional research in sex differences, age differences, and the importance of these two factors in the development of new treatments for nicotine addiction not only in schizophrenia, but also the general population.

It is also important to point out that all antipsychotics used to treat schizophrenia block the dopamine D2 receptor with some affinity. The results of this study demonstrate that the D2

and D3 receptor are involved in both the physiological and psychological aspects of nicotine addiction. The applications of these findings are very important relative to the interaction of pharmacological treatment and typical nicotine abuse in the schizophrenic population. Therapies should focus not only on pharmacologically treating the psychological addiction to nicotine but also the psychological addiction triggered by environmental cues.

REFERENCES

- Adler, L.E., Olincy, A., Waldo, M., Harris, J.G., Griffith, J., Stevens, K., et al., (1998). Schizophrenia, sensory gating, and nicotinic receptors. *Schizophrenia Bulletin*, 24, 189-202.
- Adler, L.E., Freedman, R., Ross, R.G., Olincy, A., & Walso, M.C. (1999). Elementary phenotypes in the neurobiological and genetic study of schizophrenia. *Biological Psychiatry*, 46, 8-18.
- Agassandian, K., Gedney, M., & Cassell, M.D. (2006). Neurotrophic factors in the central nucleus of the amygdala may be organized to provide substrates for associative learning [Electronic version]. *Brain Research*, 1076, 78-86.
- Akimoto, K., Hamamura, T., Kazahaya, Y., Akiyama, K., & Otsuki, S. Enhanced extracellular dopamine level may be the fundamental neuropharmacological basis of cross-behavioral sensitization between methamphetamine and cocaine—an in vivo dialysis study in freely moving rats. *Brain Research*, 507, 344-346.
- Aloe, L., Iannitelli, A., Angelucci, F., Bersani, G., & Fiore, M. (2000). Studies in animal models and humans suggesting a role of nerve growth factor in schizophrenia-like disorders. *Behavioral Pharmacology*, 11, 235-242.
- American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed. *DSM-IV* Text revision). Arlington, VA: Author.
- Andersen, S.L., & Teicher, M.H. (2000). Sex differences in dopamine receptor density and their relevance to ADHD [Review]. *Neuroscience and Biobehavioral Reviews*, 24, 137-141.

- Andia, A.M., Zisook, S., Heaton, R.K., Hesselink, J., Jernigan, T., Kuck, J., et al., (1995). Gender differences in schizophrenia. *Journal of Nervous and Mental Disease*, 183, 522-528.
- Angelucci, F., Mathe, A.A., & Aloe, L. (2004). Neurotrophic factors and CNS disorders: Findings in rodent models of depression and schizophrenia. *Progress in Brain Research*, 146, 151-165.
- Balfour, D., Wright, A.E., Benwell, M., & Birrell, C.E. (2000). The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. *Behavioral Brain Research*, 112, 73-83.
- Barde, Y.A., Davies, A.M., Johnson, J.E., Lindsay, R.M., & Thoenen, H. (1987). Brain derived neurotrophic factor. *Progress in Brain Research*, 71, 185-189.
- Barik, J., & Wonnacott, S. (2006). Indirect modulation by $\alpha 8$ nicotinic acetylcholine receptors of noradrenaline release in rat hippocampal slices: Interaction with glutamate and GABA systems and effect of nicotine withdrawal. *Molecular Pharmacology*, 2, 618-628.
- Becker, J.B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology, Biochemistry, and Behavior*, 64, 803-812.
- Becker, J.B., & Cha, J. (1989). Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. *Behavioural Brain Research*, 35, 117-125.
- Becker, J.B., Snyder, P.J., Miller, M.M., Westgate, S.A., & Jenuwine, M.J. (1987). The influence of estrous cycle and intrastriatal estradiol on sensorimotor performance in the female rat. *Pharmacology, Biochemistry, and Behavior*, 27, 53-59.

- Benowitz, N.L. (1988). Pharmacologic aspects of cigarette smoking and nicotine addiction. *The New England Journal of Medicine*, 319, 1318-1330.
- Benowitz, N.L. (2008). Neurobiology of nicotine addiction: Implications for smoking cessation. *The American Journal of Medicine*, 121, S3-10.
- Betancur, C., Lepee-Logeoux, I., Cazillis, M., Accili, D., Fuchs, S., & Rostene, W. (2001). Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D(3) receptor deficient mice. *Neuropsychopharmacology*, 24, 170-182.
- Bevins, R.A., Besheer, J., & Pickett, K.S. (2001). Nicotine-conditioned locomotor activity in rats: Dopaminergic and GABAergic influences on conditioned expression. *Pharmacology, Biochemistry, and Behavior*, 68, 135-145.
- Bevins, R. A., Eurek, S., & Besheer, J. (2005). Timing of conditioned responding in a nicotine locomotor conditioning preparation: manipulations of the temporal arrangement between context cues and drug administration [Electronic version]. *Behavioural Brain Research*, 159, 135-143.
- Bevins, R.A., & Palmatier, M. I. (2003). Nicotine-conditioned locomotor sensitization in rats: Assessment of the US-preexposure effect [Electronic version]. *Behavioural Brain Research*, 143, 65-74.
- Bigos, K.L., Pollock, B.G., Coley, K.C., Miller, del D., Marder, S.R., Aravagiri, M., et al., (2008). Sex, race, and smoking impact olanzapine exposure. *Journal of Clinical Pharmacology*, 48, 157-165.
- Bobb, A.J., Castellanos, F.X., Addington, A.M., & Rapoport, J.L. (2005). Molecular genetic studies of ADHD: 1991 to 2004. *American Journal of Medical Genetics*, 132, 109-125.

- Booze, R.M., Welch, M.A., Wood, M.L., Billings, K.A., Apple, S.R., & Mactutus, C.F. (1999). Behavioral sensitization following repeated intravenous nicotine administration: Gender differences and gonadal hormones. *Pharmacology, Biochemistry, & Behavior*, *64*, 827-839.
- Braff, D.L., & Geyer, M.A. (1990). Sensorimotor gating and schizophrenia: Human and animal model studies [Abstract]. *Archives of General Psychiatry*, *47*, 181-188.
- Brown, R.W., Gass, J.T., & Kostrzewa, R.M. (2002). Ontogenetic quinpirole treatments produce spatial memory deficits and enhance skilled reaching in adult rats. *Pharmacology, Biochemistry, and Behavior*, *72*, 591-600.
- Brown, R.W., Gonzalez, C.L.R., Whishaw, I.Q., & Kolb, B. (2001) Nicotine improvement of Morris water task performance after fimbria-fornix lesion is blocked by mecamylamine. *Behavioural Brain Research*, *119*, 185-192.
- Brown, R.W., & Kolb I. (2001). Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. *Brain Research*, *899*, 94-100.
- Brown, R.W., Perna, M.K., Maple, A.M., Wilson, T.D., & Miller, B.E. (2008). Adulthood olanzapine treatment fails to alleviate decreases of ChAt and BDNF RNA expression in rats quinpirole-primed as neonates [Electronic version]. *Brain Research*, *20*, 66-77.
- Brown, R.W., Perna, M.K., Schaefer, T.L., & Williams, M.T. (2006). The effect of adulthood nicotine treatment on D2-mediated behavior and neurotrophins of rats neonatally treated with quinpirole [Electronic version]. *Synapse*, *59*, 253-259.
- Brown, R.W., Thompson, K.D., Thompson, K.N., Thacker, S.K., Ward, J.J., & Kostrzewa R.M. (2004). Adulthood nicotine treatment alleviates behavioural impairments in rats neonatally treated with quinpirole: possible roles of acetylcholine function and

- neurotrophic factor expression. *European Journal of Neuroscience*, 19, 1634-1642.
- Brown, R.W., Thompson, K.N, Click, I.A., Best, R., Thacker, S.K., & Perna, M.K. (2005). The effects of eticlopride on Morris water task performance in male and female rats neonatally treated with quinpirole. *Psychopharmacology*, 180, 234-240.
- Cardinal, R.N., Parkinson, J.A., Hall, J., & Everitt, B.J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews*, 26, 321-352.
- Carey, R.J., DePalma, G., Damianopoulos, E., Shanahan, A., Muller, C.P., & Huston, J.P. (2005). Evidence that the 5-HT_{1A} autoreceptor is an important pharmacological target for the modulation of cocaine behavioral stimulant effects. *Brain Research* 1034, 162-171.
- Carlsson, M.L. (2002). On the role of cortical glutamate in obsessive-compulsive disorder and attention-deficit disorder, two phenomenologically antithetical conditions. *Acta Psychiatrica, Scandinavica*, 102, 401-413.
- Cassell, M.D., Freedman, L.J., & Shi, C. (1999). The intrinsic organization of the central extended amygdala: *Annals of the New York Academy of Sciences*, 877, 217-241.
- Chambers, R.A., Moore, J. McEvoy, J.P., & Levin, E.D. (1996). Cognitive effects of neonatal hippocampal lesions in a rodent model of schizophrenia. *Neuropsychopharmacology*, 15, 587-94.
- Chiang, Y.C., Chen, P.C., & Chen, J.C. (2003). D₃ dopamine receptors are down-regulated in amphetamine sensitized rats and their putative antagonists modulate the locomotor sensitization to amphetamine. *Brain Research*, 972, 159-167.

- Clarke, P.B., Fu, D.S., Jakubovic, A., & Fibiger, H.C. (1988). Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. *The Journal of Pharmacology and Experimental Therapeutics*, *248*, 701-708.
- Collins, G.T., Witkin, J.M., Newman, A.H., Svensson, K.A., Grundt, P., Cao, J., et al., (2005). Dopamine agonist-induced yawning in rats: A dopamine D3 receptor-mediated behavior. *The Journal of Pharmacology and Experimental Therapeutics*, *314*, 310-319.
- Dalack, G. W., Healy, D. J., & Meador-Woodruff, J.H. (1998). Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings [Electronic version]. *American Journal of Psychiatry*, *155*, 1490-1501.
- Davis, K.L., Kahn, R.S., Ko, G., & Davidson, M. (1991). Dopamine in schizophrenia: A review and reconceptualization. *American Journal of Psychiatry*, *148*, 1474-1486.
- Day, J., & Fibiger, H.C. (1992). Dopaminergic regulation of cortical acetylcholine release. *Synapse*, *12*, 281-286.
- Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., et al., (2004). Dopamine and drug addiction: The nucleus accumbens shell connection [Electronic version]. *Neuropharmacology*, *47*, 227-241.
- Dluzen, D.E., & Anderson, L.I. (1997). Estrogen differentially modulates nicotine-evoked dopamine release from the striatum of male and female rats [Electronic version]. *Neuroscience Letters*, *230*, 140-142.
- Domino, E.F. (2001). Nicotine induced locomotor sensitization. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *25*, 59-71.

- Donny, E.C., Caggiula, A.R., Rowell, P.P. Gharib, M.A., Maldovan, V., Booth, S., et al., (2000) Nicotine self-administration in rats: Estrous cycle effects, sex differences, and nicotinic receptor binding. *Psychopharmacology (Berlin)*, 151, 392-405.
- Durany, N., Michel, T., Zochling, R., Boissl, K.W., Cruz-Sanchez, F.F., Riederer, P., et al., (2001). Brain-derived neurotrophic and neurotrophin 3 in schizophrenic psychoses. *Schizophrenia Research*, 52, 79-86.
- Elliot, B.M., Faraday, M.M., Phillips, J.M., & Grunberg, N.E. (2005). Adolescent and adult female rats differ in sensitivity to nicotine's activity effects [Electronic version]. *Pharmacology, Biochemistry, & Behavior*, 80, 567-575.
- Elvevag, B. & Goldberg, T.E. (2000). Cognitive impairment in schizophrenia is the core of the disorder [Abstract]. *Critical Reviews in Neurobiology*, 14, 1-21.
- Everitt, B.J., Morris, K.A., O'Brien, A., & Robbins, T.W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preferences: Further evidence of limbic-striatal interactions underlying reward-related processes [Abstract]. *Neuroscience*, 42, 1-18.
- Faraday, M.M., Elliott, B.M., Phillips, J.M., & Grunberg, N.E. (2003). Adolescent and adult male rats differ in sensitivity to nicotine's activity effects [Electronic version]. *Pharmacology, Biochemistry, and Behavior*, 74, 917-931.
- Ferguson, S. S. G. (2001). Evolving concepts in G-protein coupled receptor endocytosis: The role of receptor desensitization and signaling. *Pharmacology Reviews*, 53, 1-24.
- Filip, M., Papla, I., & Czepiel, K. (2002). Role of dopamine D3 receptors in controlling the expression of cocaine sensitization in rats. *Polish Journal of Pharmacology*, 54, 687-691.

- Franklin, T.R., Napier, K., Ehrman, R., Gariti, P., O'Brien, C.P., & Childress, A.R. (2004). Retrospective study: Influence of menstrual cycle on cue-induced cigarette craving. *Nicotine and Tobacco Research, 6*, 171-175.
- Frantz, K.J., & Van Hartesveldt, C. (1999). The locomotor effects of quinpirole in rats depend on age and gender [Electronic version]. *Pharmacology, Biochemistry, and Behavior, 64*, 821-826.
- Gearon, J.S., & Bellack, A.S. (2000). Sex differences in illness presentation, course, and level of functioning in substance-abusing schizophrenia patients [Electronic version]. *Schizophrenia Research, 43*, 65-70.
- Geldwert, D., Norris, J.M., Feldman, I.G., Schulman, J.J., Joyce, M.P., & Rayport, S. (2006). Dopamine presynaptically and heterogeneously modulates nucleus accumbens medium-spiny neuron GABA synapses in vitro. *BMC Neuroscience, 7*:53.
- Geyer, M.A., Krebs-Thomson, K., Braff, D.L., & Swerdlow, N.R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: A decade in review. *Psychopharmacology, 156*, 117-157.
- Goldstein, J.M. (1988). Gender differences in the course of schizophrenia. *American Journal of Psychiatry, 145*, 684-689.
- Gonon, F. (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by d1 receptors in the rat striatum in vivo. *The Journal of Neuroscience, 17*, 5972-5978.
- Grady, S., Marks, M.J., Wonnacott, S., & Collins, A.C. (1992). Characterization of nicotinic receptor-mediated [3H] dopamine release from synaptosomes prepared from mouse striatum. *Journal of Neurochemistry, 59*, 848-856.

- Grunberg, N.E., Winders, S.E., & Wewers, M.E. (1991). Gender differences in tobacco use. *Health Psychology, 10*, 143-153.
- Gubellini, P., Pisani, A., Centonze, D., Bernardi, G., & Calabresi, P. (2004). Metabotropic glutamate receptors and striatal synaptic plasticity: Implications for neurological diseases. *Progress in Neurobiology, 74*, 271-300.
- Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.C., & Sokoloff, P. (2001). BDNF controls D3 receptor expression and triggers behavioral sensitization [Electronic version]. *Nature, 411*, 86-89.
- Gurevich, E.V., Bordelon, Y., Shapiro, R.M., Arnold, S.E., Gur, R.E., & Joyce, J.N. (1997). Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia [Abstract]. *Archive of General Psychiatry, 54*, 225-232
- Harrison, P.J., & Eastwood S.L. (2001). Neuropathological studies of synaptic connectivity in the hippocampal formation in schizophrenia. *Hippocampus, 11*, 508-519.
- Harrod, S.B., Booze, R.M., & Mactutus, C.F. (2007). Sex differences in nicotine levels following repeated intravenous injection in rats are attenuated by gonadectomy. *Pharmacology, Biochemistry, and Behavior, 86*, 32-36.
- Harrod, S.B., Mactutus, C.F., Bennett, K., Hasselrot, U., Wu, G., Welch, M., et al., (2004). Sex differences and repeated intravenous nicotine: behavioral sensitization and dopamine receptors [Electronic version]. *Pharmacology, Biochemistry, and Behavior, 78*, 581-592.
- Heresco-Levy, U., Silipo, G., & Javitt, D.C. (1996). Glycinergic augmentation of NMDA receptor-mediated neurotransmission in the treatment of schizophrenia. *Psychopharmacology Bulletin, 32*, 731-740.

- Hukkanen, J., Jacob, P., & Benowitz, N.L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacological Reviews*, 57, 79-115.
- Iizuka, Y., Sei, Y., Weinberger, D.R., & Straub, R.E. (2007). Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization. *Journal of Neuroscience*, 24, 12390-12395.
- Impey, S., Smith, D.M., Obrietan, K., Donahue, R., Wade, C., & Storm, D.R. (1998). Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nature Neuroscience*, 1, 595-601.
- Iritani, S., Niizato, K., Nawa, H., Ikeda, K., & Emson, P.C. (2003) Immunohistochemical study of brain-derived neurotrophic factor and its receptor, TrkB, in the hippocampal formation of schizophrenic brains. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 27, 801-807.
- Jarvis, M. J. (2004). Why people smoke [Electronic version]. *British Medical Journal*, 328, 277-279.
- Joseph, M.H., Datla, K., & Young A.M.J. (2003). The interpretation of the measurement of nucleus accumbens dopamine by in vivo dialysis: the kick, the craving, or the cognition? *Neuroscience and Biobehavioral Reviews*, 27, 527-541.
- Josselyn, S.A., Shi, C., Carlezon, W.A., Neve, R.L., Nestler, E.J., & Davis, M. (2001). Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. *Journal of Neuroscience*, 21, 2404-2412.
- Kabbani, N., Negyessy, L., Lin, R., Goldman-Rakic, P., & Levenson, R. Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor. *The Journal of Neuroscience*, 22, 8476-8486.

- Kalat, J.W. (2004). Chemical events at the synapse. In *Biological psychology*, (8th ed., pp. 53-72). Belmont, CA: Wadsworth.
- Kalivas, P.W., & Duffy, P. (1990). The effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse*, 5, 48-58.
- Kalivas, P.W., & Duffy, P. (1993). Time course of extracellular dopamine and behavioral sensitization of cocaine. *Journal of Neuroscience*, 13, 266-275.
- Kalivas, P.W. & Stewart J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research Reviews*, 16, 223-244.
- Kanyt, L., Stolerman, I.P., Chandler, C.J., Saigusa, T., & Pogun, S. (1999). Influence of sex and female hormones on nicotine-induced changes in locomotor activity in rats. *Pharmacology, Biochemistry, and Behavior*, 62, 179-187.
- Kelley, A.E. (2001). Measurement of rodent stereotyped behavior. *Current protocols in neuroscience*, (J.N. Crawley, Ed.). New York: J. Wiley.
- Kelley, B.M., & Rowan, J.D. (2004). Long-term, low-level adolescent nicotine exposure produces dose-dependent changes in cocaine sensitivity and reward in adult mice. *International Journal of Developmental Neuroscience*, 22, 339-348.
- Kelly, C., & McCreadle, R. G. (1999). Smoking habits, current symptoms, and premorbid characteristics of schizophrenic patients in Nithsdale, Scotland [Electronic version]. *American Journal of Psychiatry*, 156, 1751-1757.
- Kobayashi, M., Iaccarino, C., Saiardi, A., Heidt, V., Bozzi, Y., Picetti, R., et al., (2004). Simultaneous absence of dopamine D1 and D3 receptor-mediated signaling is lethal in mice. *Proceedings of the National Academy of Sciences of the United States of*

- American*, 101, 11465-11470.
- Kokkinidis, L., & Anisman, H. (1980). Amphetamine models of paranoid schizophrenia: an overview and elaboration of animal experimentation. *Psychological Bulletin*, 88, 551-579.
- Kosowski, A.R., & Liljequist, S. (2005). Behavioral sensitization to nicotine precedes the onset of nicotine-conditioned locomotor stimulation. *Behavioral Brain Research*, 156, 11-17.
- Kostrzewa, R.M. (1995). Dopamine receptor supersensitivity. *Neuroscience and Biobehavioral Reviews*, 19, 1-21.
- Kostrzewa, R.M., & Brus, R. (1991). Ontogenetic homologous supersensitization of quinpirole-induced yawning in rats. *Pharmacology, Biochemistry, and Behavior*, 39, 39-50.
- Kostrzewa, R.M., Hamdi, A., & Kostrzewa, F.P. (1990). Production of prolonged supersensitization of dopamine D2 receptors. *European Journal of Pharmacology*, 183, 42-49.
- Krupnick, J.G., & Benovic, J.L. (1998). The role of receptor kinases and arrestins in G-protein-coupled receptor regulation. *Annual Reviews in Pharmacology and Toxicology*, 32, 289-319.
- Kuczenski, R., & Segal, D.S. (2001). Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: Relative roles of dopamine and norepinephrine. *Journal of Pharmacology and Experimental Therapeutics*, 296, 876-883.
- Kumari, V., & Postma, P. (2005). Nicotine use in schizophrenia: The self medication hypotheses [Electronic version]. *Neuroscience and Biobehavioral Reviews*, 29, 1021-1034.

- Kumari, V., & Sharma, T. (2002). Effects of typical and atypical antipsychotics on prepulse inhibition in schizophrenia: A critical evaluation of current evidence and directions for future research. *Psychopharmacology*, *162*, 97-101.
- Kyerematen, G.A., Owens, G.F, Chattopadhyay, B., deBethizy, J.D., & Vesell, E.S. (1988). Sexual dimorphism of nicotine metabolism and distribution in the rat: Studies in vivo and in vitro. *Drug Metab Dispos*, *16*, 823-828.
- Lacroix, L., Broersen, L.M., Feldon, J., & Weiner, I. (2000) Effects of local infusions of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine induced activity. *Behavioural Brain Research*, *107*, 111-118
- Lapin, E.P., Maker, H.S., Sershen, H., Hurd, Y., & Lajtha, A. (1987). Dopamine-like action of nicotine: Lack of tolerance and reverse tolerance. *Brain Research*, *407*, 351-363.
- Lavalaye, J., Linszen, D.H., Booij, J., Dingemans, P.M., Reneman, L., et al., (2001). Dopamine transporter density in young patients with schizophrenia assessed with [123]FP-CIT SPECT. *Schizophrenia Research*, *47*, 59-67.
- Lazev, A. B., Herzog, T. A., & Brandon, T. H. (1999). Classical conditioning of environmental cues to cigarette smoking. *Experimental Clinical Psychopharmacology*, *7*, 56-63.
- Ledoux, J.E. (2000) Emotion circuits in the brain. *Annual Review of Neuroscience*, *23*, 155-184.
- Le Foll, B., Diaz, J., & Sokoloff, P. (2003). Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. *Synapse*, *47*, 176-183.
- Le Foll, B., Schwartz, J.C., & Sokoloff, P. (2003). Disruptions of nicotine conditioning by D(3) receptor ligands. *Molecular Psychiatry*, *8*, 225-230.
- Le Foll, B., Sokoloff, P., Stark, H., & Goldberg, S.R. (2005). Dopamine D3 receptor ligands block nicotine-induced conditioned place preferences through a mechanism that does not

- involve discriminative-stimulus or antidepressant-like effects.
Neuropsychopharmacology, 30, 720-730.
- Leonard, S., Gault, J., Hopkins, J., Logel, J., Vianzon, R., et al., (2002). Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Archives of General Psychiatry*, 59, 1085-1096.
- Lepekhina, L.M., & Tsitsurina, E.A. (2007). Stereotyped behavior in the ontogeny of rats.
Bulletin of Experimental Biology and Medicine, 144, 349-351.
- Leung, A., & Chue, P. (2000). Sex differences in schizophrenia, a review of the literature [Review]. *Acta Psychiatrica Scandinavica*, 401, 3-38.
- Levi-Montalcini, R. (1987). The neurotrophic factor: Thirty-five years later. *Bioscience Reports*, 7, 681-699.
- Lipska, B.K., Lerman, D.N., Khaing, Z.Z., & Weinberger, D.R. (2003). The neonatal ventral hippocampal lesion model of schizophrenia: effects on dopamine and GABA mRNA markers in the rat midbrain. *European Journal of Neuroscience*, 18, 3097-3104.
- Lipska, B.K., & Weinberger, D. R. (2002). A neurodevelopmental model of schizophrenia: Neonatal disconnection of the hippocampus. *Neurotoxicology Research*, 5-6, 469-475..
- Mackay, A.V., Iversen, L.L., Rossor, M., Spokes, E., Bird, E., Arregui, A., Creese, I., & Snyder, S.H. (1982). Increased brain dopamine and dopamine receptors in schizophrenia.
Archives of General Psychiatry, 39, 991-997.
- Maple, A.M., Perna, M.K., Parlaman, J.P., Stanwood, G.D., & Brown, R.W. (2007). Ontogenetic quinpirole treatment produces long-lasting decreases in the expression of RGS9 but increases in RGS17 in the striatum, nucleus accumbens, and frontal cortex [Electronic version]. *European Journal of Neuroscience*, 26, 2532-2538. .

- Marcondes, F.K., Bianchi, F.J., & Tanno, A.P. (2002). Determination of the estrous cycle phases of rats: Some helpful considerations [Electronic version]. *Brazilian Journal of Biology*, 62, 609-614.
- Mark, G.P., Kinney, A.E., Grubb, M.C., Zhu, X., Finn, D.A., Mader, S.L., et al., (2006). Injection of oxotremorene in nucleus accumbens shell reduces cocaine but not food self-administration in rats. *Brain Research*, 1123, 51-59.
- Mason, N.J., Kozell, L.B., & Neve, K.A. (2002). Regulation of dopamine D1 receptor trafficking by protein kinase A-dependent phosphorylation. *Molecular Pharmacology*, 61, 806-816.
- McKay, B.E., Placzek, A.N., & Dani, J.A. (2007). Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochemical Pharmacology*, 74, 1120-1133.
- Meredith, G.E., Callen, S., & Scheuer, D.A. (2002). Brain-derived neurotrophic factor expression is increased in the rat amygdala, piriform cortex and hypothalamus following repeated amphetamine administration [Electronic version]. *Brain Research*, 949, 218-227.
- Milesi-Halle, A., McMillan, D.E., Laurenzana, E.M., Byrnes-Blake, K.A., & Owens, S.M. (2007). Sex differences in (+)-amphetamine- and (+)-methamphetamine-induced behavioral response to male and female Sprague-Dawley rats. *Pharmacology, Biochemistry, and Behavior*, 86, 140-149.
- Millan, M.J. (2005). N-methyl-D-aspartate receptors as a target for improved antipsychotic agents: Novel insights and clinical perspectives. *Psychopharmacology*, 179, 30-53.
- Millan, M.J. (2002). N-methyl-D-aspartate receptor-coupled glycine-B receptors in the pathogenesis and treatment of schizophrenia: A critical review [Review]. *Current Drug*

- Targets, CNS and Neurological Disorders, 1*, 191-213.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., & Caron, M.G. (1998). Dopamine receptors: From structure to function. *Physiological Reviews*, *78*, 189-225.
- Mogenson, G.J., & Yang, C.R. (1991). The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. *Advances in Experimental Medicine and Biology*, *295*, 267-290.
- Murray, J. E., & Bevins, R.A. (2007). Behavioral and neuropharmacological characterization of nicotine as a conditioned stimulus. *European Journal of Pharmacology*, *561*, 91-104.
- Nowak, P., Brus, R., & Kostrzewa, R.M. (2001). Amphetamine-induced enhancement of neostriatal in vivo microdialysate dopamine content in rats, quinpirole-primed as neonates. *Polish Journal of Pharmacology*, *53*, 319-329.
- Pak, A.C., Ashby, C.R., Jr., Heidbreder, C.A., Pilla, M., Gilbert, J., Xi, Z.X., et al., (2006). The selective dopamine D3 antagonist SB-277011A reduced nicotine-enhanced brain-reward and nicotine-paired environmental cue functions. *The International Journal of Neuropsychopharmacology*, *9*, 585-602.
- Palmatier, M.I., Bevins, R.A. (2002). Examination of GABAergic and dopaminergic compounds in the acquisition of nicotine-conditioned hyperactivity. *Neuropsychobiology*, *45*, 87-94.
- Patterson, S.L., Abel, T., Deuel, T.A., Martin, K.C., Rose, J.C., & Kandel, E.R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron*, *16*, 1137-1145.
- Parikh, V., Evans, D.R., Kahn, M.M., & Mahadik S.P. (2003). Nerve growth factor in never-medicated first-episode psychotic and medicated chronic schizophrenic patients: Possible implications for treatment outcome. *Schizophrenic Research*, *60*, 117-123.

- Perna, M.K. (2006). *Nicotine sensitization in a rodent model of schizophrenia: A comparison of gender, age, and neurotrophic factors*. Unpublished master's thesis, East Tennessee State University, Johnson City, Tennessee.
- Pontieri, F. E., Tanda, G., Orzi, F., & Di Chiara, G. (1996). Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature*, *382*, 255-257.
- Poo, M. M. (2001). Neurotrophins as synaptic modulators [Electronic version]. *Nature Reviews, Neuroscience*, *2*, 24-32.
- Quarta, D., Ciruela, F., Patkar, K., Borycz, J., Solinas, M., Lluís, C., Franco, R., Wise, R.A., Goldberg, S.R., Hope, B.T., Woods, A.S., & Ferré, S. (2007). Heteromeric nicotinic acetylcholine-dopamine autoreceptor complexes modulate striatal dopamine release. *Neuropsychopharmacology*, *32*, 35-42.
- Rattiner, L.M., Davis, M., French, C.T., & Ressler, K.J. (2004). Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *The Journal of Neuroscience*, *24*, 4796-4806.
- Richtand, N.M., Goldsmith, R.J., Nolan, J.E., & Berger, S.P. (2001). The D3 receptor and substance dependence. *The Journal of Addictive Diseases*, *20*, 19-32.
- Richtand, N.M., Logue, A.D., Wlege, J.A., Perdiue, J., Tubbs, L.J., Spitzer, R.H., et al. (2000). The dopamine D3 receptor antagonist nafadotride inhibits development of locomotor sensitization to amphetamine. *Brain Research*, *867*, 239-242.
- Robinson, T.E., & Berridge, K.C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews*, *18*, 247-291.
- Rosa-Neto, P., Lou, H.C., Cumming, P., Pryds, O., Karrebaek, H., Lunding, J., et al. (2005). Methylphenidate-evoked changes in striatal dopamine correlate with inattention and

- impulsivity in adolescents with attention deficit hyperactivity disorder. *Neuroimage*, 25, 868-876.
- Salin-Pascual, R.J., Alcocer-Castillejos, N.V., & Alejo-Galarza, G. (2003). Nicotine dependence and psychiatric disorders. *Revista Investigacion Clinica*, 55, 677-693.
- Sams-Dodd, F. (1997). Effect of novel antipsychotic drugs on phencyclidine stereotyped behavior and social isolation in the rat social interaction test. *Behavioral Pharmacology*, 8, 196-215.
- Schindler, C.W. & Carmona, G.N. (2002). Effects of dopamine agonists on locomotor activity in male and female rats [Electronic version]. *Pharmacology, Biochemistry, and Behavior*, 72, 857-863.
- Seeman, P., Ko, F., Jack, E., Greenstein, R., & Dean, B. (2007). Consistent with dopamine supersensitivity, RGS9 expression is diminished in amphetamine-treated animal model of schizophrenia and in postmortem schizophrenia brain. *Synapse*, 61, 303-309.
- Segal, D.S., & Kuczenski, R. (1992). Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. *Brain Research*, 577, 351-355.
- Shatzberg, A.F., & Nemeroff, C.B. (Eds.) (1998). *Textbook of psychopharmacology* (2nd ed.). Washington, DC: American Psychiatric Press.
- Shovel, G., & Weizman, A. (2005). The possible role of neurotrophins in the pathogenesis and therapy of schizophrenia [Electronic version]. *European Neuropsychopharmacology*, 15, 319-329.
- Shim, I., Javaid, J.I., Wirtshafter, D., Jang, S.Y., Shin, H.J., Chung, Y.C., et al. (2001). Nicotine-induced behavioral sensitization is associated with extracellular dopamine release and

- expression of c-Fos in the striatum and nucleus accumbens of the rat. *Behavioral Brain Research*, *121*, 137-147.
- Shoaib, M., Benwell, M., Akbar, M.T., Stolerman, I.P., & Balfour, D. (1994). Behavioural and neurochemical adaptations to nicotine in rats: Influence of NMDA antagonists [Abstract]. *British Journal of Pharmacology*, *111*, 1073-1080.
- Shram, M.J., Funk, D., Li, Z., & Le, A.D. (2006). Periadolescent and adult rats respond differently in test measuring the rewarding and aversive effects of nicotine [Electronic version]. *Psychopharmacology*, *186*, 201-208.
- Smith, R.F. (2003). Animal models of periadolescent substance abuse [Review]. *Neurotoxicology and Teratology*, *25*, 291-301.
- Smith, K.J., Perna, M.K., & Brown, R.W. (2006). *Neonatal quinpirole treatment produces prepulse inhibition deficits in adult rats*. Manuscript submitted for publication.
- Smith, M.S., Freeman, M.E., & Neil, J.D. (1975). The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin, and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology*, *96*, 219-226.
- Sokoloff, P., Martres, M.P., Giros, B., Bouthenet, M.I., & Schwartz, J.C. (1992). The third dopamine receptor (D3) as a novel target for antipsychotics. *Biochemical Pharmacology*, *43*, 659-666.
- Soares, J.C., & Innis, R.B. (1999). Neurochemical brain imaging investigations of schizophrenia. *Biological Psychiatry*, *46*, 600-615.
- Stanwood, G.D., McElligot, S., Lu, L., & McGonigle, P. (1997). Ontogeny of dopamine D3 receptors in the nucleus accumbens of the rat. *Neuroscience Letters*, *223*, 13-16.

- Stolerman, I.P., Garcha, H.S., & Mirza, N.R. (1995). Dissociations between the locomotor stimulant and depressant effects of nicotinic agonists in rats. *Psychopharmacology*, *117*, 430-437.
- Sziraki, I., Sershen, H., Hashim, A., & Lajtha, A. (2002). Receptors in the ventral tegmental area mediating nicotine-induced dopamine release in the nucleus accumbens. *Neurochemical Research*, *27*, 253-261.
- Tenn, C.C., Kapur, S., & Fletcher, P.J. (2003). Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia. *Schizophrenia Research*, *64*, 103-114.
- Tenn, C.C., Kapur, S., & Fletcher, P.J. (2005). Sensitization to amphetamine, but not phencyclidine, disrupts prepulse inhibition and latent inhibition. *Psychopharmacology (Berlin)*, *180*, 366-376.
- Thacker, S. K., Perna, M.K., Ward, J.J., Schaefer, T.L., Williams, M.T., Kostrzewa, R. M., et al. (2006). The effects of adulthood olanzapine treatment on cognitive performance and neurotrophic factor content in male and female rats neonatally treated with quinpirole. *The European Journal of Neuroscience*, *24*, 2075-2083.
- Tilson, H.A., & Rech, R.H. (1973). Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. *Pharmacology, Biochemistry, and Behavior*, *1*, 149-153.
- Van Hartesveldt, C., Meyer, M.E., & Potter, T.J. (1994). Ontogeny of biphasic locomotor effects of quinpirole. *Pharmacology, Biochemistry, and Behavior*, *48*, 781-786.
- Vezina, P. (1996). D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. *Journal of Neuroscience*, *16*, 2411-2420.

- Venton, B.J., Zhang, H., Garris, P.A., Phillips, P.E., Sulzer, D., & Wightman, R.M. (2003). Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *Journal of Neurochemistry*, 87, 1284-1295.
- Versiani, M. (2006). Ziprasidone in bipolar disorder. *Expert Opinion on Pharmacotherapy*, 7, 1221-1228.
- Vetulani, J. (2001). Drug addiction. Part II. Neurobiology of addiction. *Polish Journal of Pharmacology*, 53, 304-317.
- Vezina, P., & Stewart, J. (1989). The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Research*, 499, 108-120.
- Waldron, I. (1999). Patterns and causes of gender differences in smoking. *Social Science & Medicine*, 32, 989-1005.
- Wise, R. A., & Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. *Psychological Reviews*, 94, 469-492.
- Wonnacott, S., Kaiser, S., Mogg, A., Soliakov, L., & Jones, I.W. (2000). Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. *European Journal of Pharmacology*, 393, 51-58.
- Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J., & Khansa, M.R. (1993). Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *The Journal of Pharmacology and Experimental Therapeutics*, 264, 249-255.
- Yamada, K., & Nabeshima, T. (2000). Animal models of Alzheimer's disease and anti-dementia drugs. *Pharmacology and Therapeutics*, 88, 93-113.

Yamamoto, J. (1997). Cortical and hippocampal EEG power spectra in animal models of schizophrenia produced with methamphetamine, cocaine, and phencyclidine.

Psychopharmacology, 131, 379-387.

VITA

ASHLEY BRIANNA SHEPPARD

- Education: B.S. Psychology, The University of Virginia's College at Wise
Wise, VA 2006
- M.A. Psychology, East Tennessee State University, Johnson City,
TN 2008
- Professional Experience: Graduate Assistant, East Tennessee State University, Department
of Psychology, 2006-2008
- Honors and Awards: Internal grant, School of Graduate Studies, East Tennessee State
University, February 2007
- Outstanding Research Contribution in Psychology, Department of
Social and Behavioral Sciences, The University of Virginia's
College at Wise, May 2006
- Conference Presentations: Sheppard, A.B., & Horton, J.E. (2006, April). *Social attitudes
concerning organ transplantation*. Poster session presented at the
annual Appalachian Student Research forum at East Tennessee
State University, Johnson City, TN.
- Sheppard, A.B., Amine, L., & Brown, R.W. (2007, April).
Nicotine-conditioned hyperactivity in a rodent model of psychosis.
Poster session presented at the annual Appalachian Student
Research Forum at East Tennessee State University, Johnson City,
TN.
- Cope, Z. A., Sheppard, A.B., Longacre, I.D., Hughes, A.B., &
Brown, R.W. (2007, April). *D2-primed rats demonstrate increased
sensitization and stereotypy after administered d-amphetamine*.
Poster session presented at the annual Appalachian Student
Research forum at East Tennessee State University, Johnson City,
TN.
- Hughes, A.B., Sheppard, A.B., Longacre, I.D., & Brown, R.W.
(2007, April). *Methylphenidate (Ritalin) exposure to adolescent
D2-primed rats eliminates the initial hypoactive response to
nicotine in adulthood*. Poster session presented at the annual
Appalachian Student Research Forum at East Tennessee State
University, Johnson City, TN.

Noel, D. M., Correll, J. A., Sheppard, A.B., & Brown, R.W. (2007, April). *Nicotine sensitization in adolescent beta-arrestin-2 knockout mice: Implications for mechanisms of nicotine addiction*. Poster session presented at the annual Appalachian Student Research Forum at East Tennessee State University, Johnson City, TN.

Cope, Z.A., Sheppard, A.B., Longacre, I.D., Perna, M. K., Thompson, K. N., Roane, D.S., et al. (2007, October). *Amphetamine sensitization in a rodent model of psychosis*. Poster session presented at the annual meeting for the Society for Neuroscience, San Diego, CA.

Sheppard, A.B., Amine, L., Woodruff, M. L. & Brown, R.W. (2007, November) *Nicotine-conditioned hyperactivity in male and female adolescent D2-primed rats*. Poster session presented at the annual meeting for the Society for Neuroscience, San Diego, CA.

Hughes, A.B., Sheppard, A.B., Longacre, I.D., & Brown, R.W. (2007, November). *Methylphenidate locomotor sensitization in adolescent D2-primed rats: Effects of exposure on initial nicotine activity in adulthood*. Poster session presented at the annual meeting for the Society for Neuroscience, San Diego, CA.

Sheppard, A.B., Amine, Woodruff, M.L. & Brown, R.W. (2008, January). *Nicotine-conditioned hyperactivity in adolescent D2-primed rats*. Poster session presented at the Appalachian Chapter of Society for Neuroscience Spring meeting, Johnson City, TN.

Hughes, A.B., Sheppard, A.B. Longacre, I.D., & Brown, R.W. (2008, January). *Methylphenidate locomotor sensitization in adolescent D2-primed rats*. Poster session presented at the Appalachian Chapter of the Society for Neuroscience Spring meeting, Johnson City, TN.

Cope, Z.A., Sheppard, A.B., Longacre, I.D., Perna, M.K., Thompson, K.N., Roane, D.S., et al. (2008, January). *Amphetamine sensitization in D2-primed rats*. Poster session presented at the Appalachian Chapter of Society for Neuroscience Spring meeting.

Hughes, B.A., Hughes, A.B., Sheppard, A.B., & Brown, R.W. (2008, March). *Dose-response analysis of methylphenidate locomotor sensitization in adolescent D2-primed rats*. Poster session presented at Synapse, Charleston, SC.

Lehmann, J., Sheppard, A.B., Amine, L., & Brown, R.W. (2008, March). *Nicotine-conditioned hyperactivity in male and female D2-primed adolescent rats*. Poster session presented at Synapse, Charleston, SC.

Whittemore, J.D., Cope, Z.A., Sheppard, A.B., Longacre, I.D., Perna, M.K., Thompson, K.N., et al. (2008, March). *Amphetamine sensitization in a rodent model of psychosis*. Poster session presented at Synapse, Charleston, SC.

Lehmann, J., Sheppard, A.B., Amine, L., Baisden, R.H., & Brown, R.W. (2008, November). *The role of dopamine D2 and D3 receptors in adolescent nicotine sensitization and conditioned hyperactivity in rats D2-receptor primed as neonates*. Poster session presented at the annual meeting for the Society for Neuroscience, Washington, DC.

Thompson, K. N., Cope, Z.A., Sheppard, A.B., Roane, D.S., & Brown, R.W. (2008, November). *Dopamine D2 receptor priming enhances locomotor activation and dopamine release in the nucleus accumbens core in response to amphetamine*. Poster session presented at the annual meeting for the Society for Neuroscience, Washington, DC.

Hughes, B.A., Hughes, A.B., Sheppard, A.B., Pond, B.B., Woodruff, M. L., & Brown, R.W. (2008, November). *A dose response analysis of methylphenidate sensitization in adolescent rats: Sex differences in locomotor activation*. Poster session presented at the annual meeting for the Society for Neuroscience, Washington, DC.

Noel, D.M., Hughes, B.A., Sheppard, A.B., Ordway, G. A., & Brown, R.W. (2008, November). *Eszipiclone facilitation of the antidepressant efficacy of fluoxetine using a social defeat stress model in the mouse*. Poster session presented at the annual meeting for the Society for Neuroscience, Washington, DC.