8-2007

The Effects of Nicotine Conditioned Place Preference in D2 Primed Adolescent Rats: Age-Related and Gender Effects.

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A thesis

presented to

the faculty of the Department of Psychology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Arts in Psychology

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by

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August 2007

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Keywords: Adolescence, Early Postweanling Age, Conditioned Place Preference (CPP), D_2 Receptor Supersensitization, Elevated T-Maze (ETM), Nicotine, Quinpirole, Schizophrenia

ABSTRACT

The Effects of Nicotine Conditioned Place Preference in D_2 Primed Adolescent Rats: Age-Related and Gender Effects

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This study investigated nicotine conditioned place preference (CPP) in two different ages of adolescence using a rodent model of schizophrenia. Both 2- and 3-chambered CPP apparatuses were used to test whether the CPP was due to an aversion to the white chamber. Animals were neontally treated with the dopamine D_2/D_3 agonist, quinpirole, or saline and raised to either early postweanling age (P 22) or adolescence (P 29). Rats were conditioned to prefer the white chamber using nicotine. Results showed that nicotine induced CPP and appeared to alleviate an increased stress response in D_2 primed animals, which appeared to diminish over time. Additionally, adult D_2 and non-D_2 primed rats were tested on the elevated T-maze. Results revealed that D_2 primed rats demonstrated a significant increase in unconditioned fear. This study showed that nicotine induced CPP in D_2 and non-D_2 primed rats regardless of age, and D_2 primed rats appear to demonstrate an increase in stress levels that was alleviated by nicotine.

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CHAPTER 1

INTRODUCTION

Schizophrenia

Schizophrenia is a widely known chronic and severe brain disorder characterized by hallucinations, delusions, cognitive deficits, and behavioral impairments. The term schizophrenia was first coined by Eugene Bleuler in 1911, who concluded that a mental dysfunction caused schizophrenia (Wilson, 2003). After Bleuler's initial identification of schizophrenia, it became a well-known mental disorder all over the world. Journals and articles began to widely use the term schizophrenia in 1949 (Cohen, 1949; Gottfried & Willner, 1949; Lipton, 1949; Torkildsen, 1949).

Current statistical data indicates that approximately 2.4 million American adults, or about 1.1 % of the population age 18 and older suffer from schizophrenia (National Institute of Mental Health, 2006). The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [DSM-IV], has reported that schizophrenia occurs equally in both men and women (American Psychiatric Association [APA], 1994). However, the estimated age of onset for men and women differs. For men, it is in the late teens to early 20s, and for women, the typical onset is in the 20s to early 30s (APA).

In order to be diagnosed, schizophrenic patients must exhibit two or more of the following characteristic symptoms for at least a month: delusions, hallucinations, disorganized speech or behavior, blunted mood, and apathy (APA, 1994). These characteristic symptoms are separated into subtypes called positive and negative symptoms. Positive symptoms are behaviors including hallucinations, delusions, and disturbed thinking, while negative symptoms are behaviors including apathy, blunted

mood, and poverty of speech (APA). Because of these symptoms, patients with schizophrenia are usually behaviorally impaired and experience social dysfunction (Ujike & Morita, 2004). As a result, they usually require social support to live, such as consistent treatment with mental health providers, or living in temporary housing managed by the government or non-profit organizations.

Recent clinical studies on schizophrenic patients have revealed that the disease results in significant structural brain abnormalities (Ferrari, Kimura, Nita, & Elkis, 2006; Hulshoff Pol et al., 2002; Nugent et al., 2007; Velakoulis et al., 2006; Yamada et al., 2007). Through magnetic resonance imaging (MRI) and computed tomography (CT), it has been shown that schizophrenics have significantly reduced cerebral and prefrontal gray matter, prefrontal white matter and enlarged lateral and third ventricles, as well as increased peripheral cerebrospinal fluid volumes compared to control groups (Ferrari et al.; Hulshoff Pol et al.). Also, findings have shown that the hippocampal tissue volumes of schizophrenic patients are significantly reduced, which is significant because the hippocampus is known to play an important role in cognitive impairment observed in the disorder (Breier et al., 1992; Gothelf et al., 2000; Nugent et al.; Velakoulis et al.). Furthermore, the volumes of the prefrontal cortical grey matter and the amygdala have been shown to be smaller compared to control subjects (Breier et al.; Premkumar, Kumari, Corr, & Sharma, 2006). These significant structural abnormalities found in the brain are thought to be responsible for many of the exhibited behavioral symptoms of patients with schizophrenia.

Possible Causes of Schizophrenia

NMDA Receptor Hypofunction Hypothesis.

Several hypotheses have been posited to describe the possible underlying mechanisms of schizophrenia. One hypothesis has focused on hypofunction of the glutamatergic N-methyl-D-asparate (NMDA) receptor, commonly called the NMDA receptor hypofunction hypothesis. This hypothesis originates from a clinical study using an NMDA receptor antagonist, phencyclidine (PCP) (Luby, Cohen, Rosenbaum, Gottlieb, & Kelly, 1959). Luby et al. revealed that PCP produces similar symptoms to those observed in schizophrenics. Similarly, Holcomb, Lahti, Medoff, Cullen, and Tamminga (2005) conducted a clinical study on schizophrenic and non-schizophrenic patients to measure blood flow through positron emission tomography (PET). They injected a NMDA receptor antagonist, ketamine, to both groups and found that patients with schizophrenia exhibited increased blood flow in the anterior cingulate cortex when compared to non-schizophrenic subjects. NMDA receptors are reported to be involved in synaptic activity of the anterior cingulate cortex, which is responsible for cognition (Liauw, Wang, & Zhuo, 2003). Therefore, it can be concluded that glutamate transmissions of NMDA receptors in schizophrenics are more sensitive to NMDA receptor antagonists. Furthermore, Holcomb et al. found that all of the subjects obtained higher scores in the Brief Psychiatric Rating Scale when blood flow in the anterior cingulate cortex increased. In other words, a decrease in NMDA receptor function in the anterior cingulate cortex produces psychotic symptoms. Therefore, NMDA receptor hypofunction in the anterior cingulate cortex may be involved in schizophrenia.

Cannabinoid Hypothesis. Two other models have been used to analyze neurochemical and behavioral mechanisms of schizophrenia. The cannabinoid hypothesis posits that schizophrenia results in part from dysfunction of the endogeneous cannabinoid system, which is caused by cannabis sativa (marijuana) use. The endogeneous cannabionoid system is involved in anxiety, fear, attention, memory, learning, and inhibitory regulatory mechanisms (Chhatwal & Ressler, 2007; Solowij & Michie, 2007). This hypothesis is based on the clinical observation that the heavy use of marijuana produces schizophrenia-like symptoms including paranoia, depersonalization, derealization, and deficits in perceptual processes (Favrat et al., 2005; Leweke, Schneider, Thies, Munte, & Emrich, 1999). Skosnik, Krishnan, Aydt, Kuhlenshmidt, and O'Donell (2006) performed a clinical study on current marijuana users. They found deficits in the electroencephalogram (EEG) neural synchronization and early-stage sensory function in marijuana users. In addition, marijuana users exhibited schizotypal personality characteristics significantly more than controls (Skosnik et al.). Furthermore, De Marchi et al. (2003) revealed that the amount of endocannabinoid anandamine in the blood was higher in schizophrenic patients than the control group. This increased endocannabinoid anandamine causes dysfunction in the peripheral and the central nervous system including hallucinations and cognitive deficits (De Marchi et al.). Thus, these two clinical studies suggest that the cannabinoid system may be involved in the development of schizophrenia.

The Diathesis-Stress Hypothesis. The diathesis-stress model posits that schizophrenia is caused by stressful environments, stressful life experiences, and genetic deficits that produce increased stress sensitivity in humans (Rosenthal, 1970). The

hypothalamic-pituitary-adrenal (HPA) axis, which releases cortisol, is activated under stressful conditions in humans. Increased cortisol levels eventually over-activate the mesolimbic dopamine system and cause a significant release of dopamine in the brain (Walker & Diforio, 1997). This increased dopamine level is thought to control positive symptoms of schizophrenia (Carlsson & Lindqvist, 1963). A clinical study on schizophrenia and healthy patients done by Yilmaz et al. (2007) showed that the cortisol level in schizophrenic patients is higher than in healthy individuals. Furthermore, they found that the longer the duration of the disease, the higher the cortisol level in schizophrenic patients (Yilmaz et al.). Interestingly, D'Souza et al. (2004) showed a relationship between the major psychoactive component of marijuana, delta-9-tetrahydrocannabinol (Delta-9-THC), stress, and schizophrenia. Healthy individuals injected with Delta-9-THC exhibited increased cortisol levels and schizophrenia-like symptoms, including cognitive dysfunctions and increased anxiety levels (D'Souza et al.). According to these studies, both stress and marijuana use may play a role in schizophrenia.

Dopamine Hyperfunction Hypothesis. One of the more researched hypotheses of schizophrenia is the dopamine hyperfunction hypothesis. Carlsson and Lindqvist (1963) found that schizophrenic patients suffer from increased dopamine levels, which is thought to be involved in the positive symptoms of schizophrenia. Cocaine and amphetamine are found to increase dopaminergic activity. Amphetamine and cocaine users exhibited schizophrenia-like symptoms, believed to be produced by their increased dopaminergic activity (Berger, 1981). The classic study that demonstrated the involvement of dopamine in schizophrenia was published by Randrup and Munkvad in 1967. In this study on

schizophrenic patients, they showed that dopamine agonists that activate the activity of dopamine receptors produce schizophrenia symptoms. On the other hand, dopamine antagonists that block the action of dopamine receptors have been shown to reduce positive symptoms in schizophrenic patients (Randrup & Munkvad).

Increased dopaminergic activity in schizophrenics may be due to their significantly increased density of dopamine D_2 receptors compared to control subjects (Pearce, Seeman, Jellinger, & Tourtellotte, 1990; Seeman, 1985). This finding indicates increased neural activity in response to a normal amount of released dopamine (Wilson, 2003). A study in patients with schizophrenia revealed increased presynaptic activity of dopamine neurons at D_2 receptors in the striatum (Abi-Dargham et al., 2000). In addition, through PET, Seeman and Niznik (1990) found that D_2 receptor density in patients with schizophrenia is increased in postmortem brain putamen and caudate nucleus. Therefore, there is an increased density of D_2 receptors, which helps to explain why schizophrenic patients may have increased sensitivity to dopamine (Seeman et al., 2005). However, one caveat to these studies is that all schizophrenics are typically medicated with antipsychotic drugs, which all block the dopamine D_2 receptor with some affinity, and are known to increase the dopamine levels and the number of dopamine D2 receptors (Risch, 1996; Seeman, 2006; Seeman, Corbett, & Van Tol, 1997; Seeman, Lee, Chau-Wong, & Wong, 1997 as cited in Kapur, Zipursky, Jones, Remington, & Houle, 2000; Tollefson, 1996).

Substance Abuse

Substance Abuse and Schizophrenia

The rate of substance abuse is one of the most serious public health problems that most nations are struggling to reduce because it easily affects communities. One group of people who are at an especially high risk for abusing substances is schizophrenia patients. It has been reported that there are higher rates of substance abuse among schizophrenics compared to the normal population (LeDuc & Mittleman, 1995). Drugs that are used in this population include cannabis, cocaine, and nicotine (Winklbaur, Ebner, Sachs, Thau, & Fischer, 2006). Schizophrenic patients are two to five times more likely to use psychoactive drugs than non-schizophrenics (LeDuc & Mittleman). Some studies have reported that abuse of psychoactive substances in the schizophrenic population produces increased memory impairment, isolation, early life stress, and depressive symptoms (Scheller-Gilkey, Thomas, Woolwine, & Miller, 2002; Sevy, Kay, Opler, & van Praag, 1990).

Nicotine

Nicotine is one of the substances most commonly abused by people who are diagnosed with mental disorders including depression, anxiety disorder, panic disorder, eating disorder, and schizophrenia (Anzengruber et al., 2006; Breslau, 1995; Hughes, Hatsukami, Mitchell, & Dahlgren, 1986; Isensee, Wittchen, Stein, Hofler, & Lieb, 2003). It is a reinforcing drug that increases dopaminergic activity in the nucleus accumbens (Miyasato, 2001). Due to these properties, nicotine is highly addictive, and people become nicotine-dependent rapidly. Withdrawal typically produces increased anxiety (Nerin, Beamonte, Gargallo, Jimenez-Muro, & Margueta, 2007). The DSM-IV

recognizes nicotine as an addictive substance that is abused by the population (APA, 1994). Small rapid doses of nicotine can be delivered to the brain within a few seconds, and nicotine binds to nicotinic acetylcholine receptors (nAChRs), which are found in both the peripheral and the central nervous system (Goodman, 1995 as cited in Uneri, Tural, & Cakin Memic, 2006; Uneri et al.). Nicotinic acetylcholine receptors are reported to be highly concentrated in the hippocampus, where they regulate glutamate release, and also in the ventral tegmental area (VTA), where they play a role in regulating dopamine release (Fallon & Loughlin, 1995; Vizi & Kiss, 1998). The hippocampus plays a major role in cognition, and the VTA sends projections to the nucleus accumbens and plays a major role in positive reinforcement and drug addiction. In addition, Cao, Surowy, and Puttfarcken (2005) found that nAChRs are responsible for dopamine release in the hippocampus. Furthermore, nAChRs are reported to control several neurotransmitter levels including norepinephrine, GABA, and acetylcholine in several brain areas (Clarke & Reuben, 1996; Wilkie, Hutson, Sullivan, & Wonnacott, 1996; Yang, Criswell, & Breese, 1996). Finally, nAChRs are found in high concentrations at the neuromuscular junction in the periphery, which indicates that they play a role in motor function (Bernheim, Hamann, Liu, Fischer-Lougheed, & Bader, 1996). Therefore, nAChR dysfunction is thought to be involved in neurodegeneration and cognitive deficits of schizophrenia.

Nicotine Exposure in Schizophrenia. There are two groups of people who are at higher risk of being nicotine dependent. It has been reported that between 74 % and 92 % of the schizophrenic population in America is nicotine dependent (de Leon et al., 1995; Hughes et al., 1986; O'Farrell, Connor, & Upper, 1983). The reason for the high rate of

nicotine abuse in this population is thought to be for self-medication purposes and to alleviate symptoms of the disorder (Araki, Suemaru, & Gomita, 2002; Krystal et al., 2006; Kumari & Postma, 2005; Punnoose & Belgamwar, 2006). Several studies have supported this explanation. Nicotine has been shown to alleviate several commonly exhibited symptoms of schizophrenia such as cognitive impairment, processing of auditory stimuli, and anxiety (Larrison-Faucher, Matorin, & Sereno, 2004; Lyon, 1999; Salas, Pieri, Fung, Dani, & De Biasi, 2003). Furthermore, Zhang et al. (in press) found that positive symptoms are alleviated in smoking schizophrenics compared to non-smoking schizophrenics, and negative symptoms are more fully alleviated the more cigarettes patients smoke.

Nicotine Exposure in Adolescence. Another group of people who are at higher risk of abusing nicotine is adolescents. A report has shown that 42 % of middle and high school students have experimented with smoking (U.S. Department of Health and Human Services, 1993 as cited in Wang et al., 1999). In addition, Stanton (1995) conducted a longitudinal study using 937 adolescents as subjects and found that 19.3 % of the subjects met the criteria for being nicotine dependent. Several findings have shown that smokers who develop their habits in adolescence have a lesser likelihood of quitting smoking later in life (DiFranza et al., 2000; Smith et al., 2006). These findings are supported by other studies that indicate adolescent rats have a higher sensitivity to nicotine than adult rats (Belluzzi, Lee, Oliff, & Leslie, 2004; Rezvani & Levin, 2004). Therefore, it is believed that adolescence may be the most critical time in developing nicotine dependence.

Rodent models of adolescent nicotine exposure have revealed that nicotine exposure causes long-term changes in the central nervous system including dopaminergic

and catecolaminergic functioning and damage in the hippocampus, midbrain, and cerebral cortex (Abreu-Villaca, Seidler, Tate, & Slotkin, 2003; Trauth, Seidler, Ali, & Slotkin, 2001). Trauth et al. found that nicotine infusion from P 30 to P 47.5 activated midbrain catecholaminergic pathways, resulting in increased dopamine metabolism. However, during the post-infusion period (P 50 to P 60) midbrain catecholamine turnover decreased in male rats. On P 80, midbrain catecholaminergic pathways were activated again in males (Trauth et al.). In another study, Abreu-Villaca et al. chronically injected three different doses of nicotine (0.6, 2, and 6 mg/kg/day), and all of these doses resulted in a decreased density, number, and size of cells, meaning that nicotine produced cell death. Nicotine decreased neuron projection in the midbrain, hippocampus, and cerebral cortex, and these brain alterations were still observed 1 month after nicotine treatment. On the other hand, 6 mg/kg/day of nicotine injection in adult rats did not produce any brain alterations (Abreu-Villaca et al.). In conclusion, these critical neuronal damages and changes caused by nicotine are thought to be key elements in causing lifetime nicotine addiction in adolescents.

Sex Difference in Adolescent Nicotine Exposure. There are some reports indicating that there may be sex differences in adolescent nicotine exposure. Piko, Wills, and Walker (in press) used 1,225 U. S. high school students as participants to analyze whether sex differences exist in cigarette smoking. The results showed that there is no significant difference between the rate of smoking in boys and girls. However, around 15 years ago, it was reported that the smoking rate was higher among boys (Johnston, O'Malley, & Bachman, 2000 as cited in Piko et al.). Piko et al. have concluded that the rate of smoking among girls has increased since that time, and the currently equal

occurring rates of smoking are due to increased acceptance of female smoking in public places.

In contrast to Piko and colleagues' study, Stanton, McClelland, Elwood, Ferry, and Silva (1996) found gender differences in the number of adolescent daily smokers (15 to 18 year-olds) in New Zealand in a longitudinal study. They found that the daily smoking rate increased from 15 % (15 year-olds) to 31 % (18 year-olds). Among girls, there was a greater number of daily smokers than boys. Two factors can possibly explain the reasons for these sex differences. Perkins, Donny, and Caggiula (1999) reported that males are more sensitive to the reinforcing effect of nicotine. Furthermore, Crisp, Sedgwick, Halek, Joughin, and Humphrey (1999) found that teenage females have higher social anxiety from weight gain and body image. Females are shown to be more sensitive to the anxiolytic effect of nicotine than males. Therefore, Crisp et al. concluded that teenage girls smoke in order to receive the calming and anxiolytic effects of nicotine.

Sex differences have been shown in the behavioral response to nicotine in adolescent mice (Klein, Stine, Vandenbergh, Whetzel, & Kamens, 2004). Mice were given 24 hour access to either saccharin solution or one of the following amounts of freebase nicotine: 10 ug/ml, 25 ug/ml, 50 ug/ml, 75 ug/ml, 100 ug/ml, or 200 ug/ml dissolved in saccharin solution. The results showed that there is no sex difference in serum cotinine (a nicotine metabolite) levels, according to the liver weights of male and female mice. However, female adolescent mice consumed more nicotine than males. The authors reasoned that this sex difference is observed due to one of two possible reasons: either female mice are less sensitive to nicotine than males, or females metabolize nicotine more quickly than males. Therefore, Klein et al. concluded that the rate of

nicotine metabolism, absorption, distribution, and excretion processes among females and males may be different.

Neuroanatomically, there are sex differences in response to nicotine. Xu, Seidler, Ali, Slikker, and Slotkin (2001) found that chronic administration of nicotine produces suppression in 5-HT activity and damage to 5-HT projection in adolescent rats. In addition, Xu, Seidler, Cousins, Slikker, and Slotkin (2002) found that female adolescent rats exhibited reduced 5-HT receptor binding due to nicotine in the cerebral cortex. Furthermore, Viveros, Marco, and File (2006) reported that females exhibited a greater change in 5-HT receptors than males that may have contributed to 5-HT receptor dysregulation. This 5-HT receptor change in adolescent females may have contributed to sex differences in behavioral responses to nicotine.

To sum up these studies, there may be sex differences in nicotine consumption and behavioral responses to nicotine in adolescence because females may be less sensitive to nicotine, and nicotine damages 5-HT functions more in females. However, there are only a limited number of articles indicating sex differences in adolescent animals. Thus, the explanations for why there may be sex differences in behavioral response to nicotine and nicotine consumption are still unclear. Further research on gender differences in nicotine exposure is needed.

Animal Models of Disease and Disorder

The use of animal models is vital for scientific research because it allows for the possibility of finding interventions or treatments for certain diseases and also a better understanding of how they develop in humans. Animal models have been shown to be especially useful in the development of novel medications and manipulations to help treat

disorders. According to McKinney and Bunney (1969), in order to validate an animal model of disease, there are three criteria that need to be reached: the similarity of conditions, behavioral states, and common mechanisms. These are all critical if an animal model is said to be valid for a particular disorder. However, even if all of the criteria are not met, and only one criterion is met, the information gathered from using the "invalid" animal model can still be useful (Woodruff & Baisden, 1994). Use of animal models met with any of the criteria raised by McKinney and Bunney allows analysis mechanisms that may be important for the progression of disease. In addition, the animal models can be used to see whether treatment works for those diseases before administering them to human subjects (Wilson, 2003).

Another reason is that animal models of disease can be correlative, analogous, or homologous to the diseases or symptoms observed in humans (Gage, Bjorklund, Isacson, & Brundin, 1985). For example, untreated animals used in a correlative animal model behave like they have been given certain drug treatments. In other words, the correlative animal model can duplicate the conditions that various different drugs would have on humans. Animals used in an analogous animal model are given lesions in the central nervous system to model certain neuropathologies that occur in the particular targeted disorder in humans. In this way, the analogous animal model can produce neurodegenerative disorders in animals such as Parkinson's disease, Alzheimer's disease, and schizophrenia, which normally only occur in humans. Finally, animals used in a homologous animal model exhibit all aspects of the disease as humans (Gage et al.). In other words, a homologous animal model is a complete duplication of human disease in

animals. Therefore, the use of animal models is very important for humans' physical and mental health.

Several animal models of disease have been produced, especially for mental disorders such as schizophrenia, major depressive disorder, anxiety disorders, and alcohol and drug addiction. Hitzemann (2000) explained that animal models of mental disorders could be used across several different behavioral tasks that accurately test observed dysfunctions of the disease and may help to identify how mental disorders are involved in particular human behaviors. Scientists primarily use animal models of mental disorders to explore possible treatments or therapeutic approaches, and certain aspects of mental disorders can be easily modeled in rodents (Hitzemann).

Rodent Models of Schizophrenia

Amphetamine Model of Schizophrenia.

Several rodent models such as the amphetamine model, PCP model and neonatal hippocampal lesion model have been used to model schizophrenia symptoms in rodents. The oldest rodent model of schizophrenia that has been prevalently used was created by the acute administration of amphetamine, which has been reported to increase dopamine release (Leyton et al., 2002; Oswald et al., 2005). This model is designed to produce analogous symptoms to the disease by producing a hyperactive dopaminergic system. The reason this model has been questioned is that the large dose administration of amphetamine in rats creates a temporally robust dopamine release, and the dopamine system is hyperactive throughout the lifetime of schizophrenics. Castner, Vosler, and Goldman-Rakic (2005) circumvented this problem by analyzing the effects of chronic administration of amphetamine on dopamine levels and behavior over a 6-week period in monkeys. They found that a

chronic administration of amphetamine produces symptomology that is similar to the positive and negative symptoms of schizophrenia, which is thought to be a result of a decreased level of dopamine turnover in the prefrontal cortex and striatum (Castner et al.). Furthermore, Haber et al. (1981) found that after several low doses of amphetamine were administered, monkeys spent a longer period of time in "sit tense" postures and showed an increased number of agonistic behaviors as well as an increased frequency of reorienting their position.

PCP Model of Schizophrenia. Another well used rodent model of schizophrenia is based on the pharmacological action of the drug PCP. The PCP model of schizophrenia is based on chronic administration of PCP, which is an antagonist at the NMDA receptor (glutamatergic receptor) and produces locomotor hyperactivity, cognitive deficits, prepulse inhibition (PPI) impairment, and impairment in social novelty discrimination in rodents (Harich, Gross, & Bespalov, 2007; Nabeshima et al., 2006; Rasmussen, O'Neil, Manaye, Perry, & Tizabi, 2007; Sams-Dodd, 1998). Schizophrenics also demonstrate significant hypoactivity at the NMDA receptor. Additionally, behaviors exhibited under the influence of PCP are similar to the positive and negative symptoms of schizophrenia. For example, rats chronically administered PCP demonstrate schizophrenic symptoms such as stereotypical behavior and social isolation (Sams-Dodd, 1996). Also, chronic PCP administration reduces N-acetylaspartate and increases N-acetylaspartylglutamate, which are both thought to be involved in fluid balance and energy production (L. M. Reynolds, Cochran, Morris, Pratt, & G. P. Reynolds, 2005).

Furthermore, rats treated with PCP also exhibit deficits in the central oxytocinergic system, which is thought to play an important role in social interaction

deficits observed in schizophrenia (Lee, Brady, Shapiro, Dorsa, & Koenig, 2005). Because these observed symptoms from PCP administration are hypothesized to be produced due to insufficient glutamate transmission, several studies have analyzed the effect of chronically administered PCP in rodents to model behavioral deficits observed in schizophrenia using this model (Harich et al., 2007; Nabeshima et al., 2006; Wass et al., 2006). Nabeshima et al. reported that decreased glutamate transmission caused by PCP administration resulted in cognitive impairments in mice tested in the Morris water maze (MWM). The primary criticism of this rodent model of schizophrenia is that the PCP model requires chronic administration to be effective. The effects of chronic PCP administration are temporary in that the behavioral deficits that are consistent with schizophrenia are demonstrated only when the drug is in the bloodstream (Sams-Dodd, 1996).

Neonatal Hippocampal Lesion Model of Schizophrenia. The most well-studied rodent model of schizophrenia is the neonatal hippocampal lesion model. One of the major differences observed through functional imaging studies among schizophrenics and non-schizophrenics is that in schizophrenics there is decreased volume of the hippocampus and frontal cortex compared to healthy subjects (Breier et al., 1992; Nugent et al., 2007; Tanskanen et al., 2005; Toulopoulou et al., 2004; Velakoulis et al., 2006; Yamasue et al., 2004). Hippocampal dysfunction presumably plays an important role in cognitive impairment and deficits in social interaction known to be present in schizophrenia (Sprick, von Wilmsdorff, Bouvier, Schulz, & Gaebel, 2006). Therefore, the purpose of this model is to try to replicate the hippocampal neuropathology that occurs in schizophrenia.

To create neonatal hippocampal lesions, rats are administered ablation of the ventral hippocampus (VH) on P 7. This type of lesion produces an increase in mesolimbic dopamine transmission (Wan, Giovanni, Kafka, & Corbett, 1996). Results have shown that lesioned rats exhibit a significant increase in activity after saline injection as well as after d-amphetamine treatment as adults. More importantly, they have reported that rats neonatally given VH lesions demonstrated increased sensitivity to stress compared to the group that was VH lesioned in adulthood (Lipska, Jaskiw, & Weinberger, 1993). This observed difference may be due to increased dopamine transmission (Lipska et al.). Also, Becker, Grecksch, Bernstein, Hollt, and Bogerts (1999) have found that neonatally lesioned rats exhibited less time in social interaction and increased aggressive behaviors. Furthermore, it is reported that neonatal hippocampal lesions produce memory and cognitive deficits in rats (Lipska, Aultman, Verma, Weinberger, & Moghaddam, 2002; Chambers, Moore, McEvoy, & Levin, 1996).

These behavioral changes are strengths of the model. On the other hand, there are a few weaknesses found in the model as well. In schizophrenia, observed brain abnormalities and neurochemical changes are due to alteration of connectivity; however, in this model these changes are produced by cell death. Also, behavioral changes produced by VH lesions differ depending on the age of the rodents (Wood, Lipska, & Weinberger, 1997).

Dopamine D_2 Receptor Priming Model of Schizophrenia. A final model of schizophrenia is one that has been created in our laboratory. Quinpirole is a dopamine D_2/D_3 agonist when it is neonatally administered. It has been shown to produce long-term supersensitization of the dopamine D_2 receptor (referred to as dopamine D_2 receptor

priming) (Kostrzewa, 1995; R. M., Kostrzewa, J. P., Kostrzewa, Nowak, R. A. Kostrzewa, & Brus, 2004; Brown et al., 2004a).

Several consistent features have been found between the neonatal quinpirole model of schizophrenia and data from human schizophrenia literature. First, amphetamine administration to adult rats neonatally treated with quinpirole produced a robust increase in the release of dopamine in the striatum (Nowak, Brus, & Kostrzewa, 2001). Consistently, studies using MRI and PET have shown that amphetamine administration produces a large increase in dopamine release in the striatum of schizophrenic patients (Lavalaye et al., 2001; Soares & Innis, 1999).

Second, neonatal quinpirole treatments have been shown to produce deficits in auditory sensorimotor gating using PPI (Smith, Perna, & Brown, n.d.). 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, which is often disrupted in schizophrenia (Kumari & Sharma, 2002; Geyer et al., 2001). It has been shown that adult rats that received neonatal quinpirole treatment demonstrated PPI deficits compared to controls using different prepulse auditory intensities (73, 76, and 82 dB) and different interstimulus intervals between the prepulse and pulse (50, 100, and 150ms) (Smith et al.).

Third, neonatal quinpirole treatments have been shown to produce long-term cognitive impairment (Brown, Gass, & Kostrzewa, 2002; Brown et al., 2004a; Brown et al., 2004b; Brown, Perna, Schaefer, & Williams, 2006). It has been well-documented that severe cognitive impairments are present in schizophrenia and suggested that cognitive impairment is a core feature of the disorder (Adler et al., 1998; Adler, Freedman, Ross,

Olincy, & Waldo, 1999; Elvevag & Goldberg, 2000). Cognitive deficits have also been hypothesized to be associated with sensorimotor gating in patients with schizophrenia (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001).

Fourth, chronic treatment with the atypical antipsychotic, olanzapine, (Trade name: Zyprexa) has been shown to alleviate cognitive deficits and long-term priming of the D_2 receptor, which is produced by neonatal quinpirole treatment. Thacker et al. (2006) have shown that chronic olanzapine treatment given twice daily in adulthood alleviated cognitive deficits produced by neonatal quinpirole treatment. Yawning is a behavior mediated by the D_2 receptor and produced by neonatal quinpirole treatment. Importantly, this treatment also alleviated the significant increase in yawning to control levels, essentially reversing the D_2 priming effect. These data demonstrate that not only is D_2 priming likely primarily responsible for these behavioral effects, but also that antipsychotic treatments are effective in alleviating these effects. In vitro analyses showed that significant decreases in nerve growth factor (NGF) in the hippocampus that was produced by neonatal quinpirole treatment, was reversed by olanzapine, and brain tissue was not taken until after an 8-day washout (Thacker et al.).

Fifth, neonatal quinpirole treatments in rats have been shown to 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 significant decreases in NGF expression in the hippocampus compared to saline controls in both early postweanling and adult rats (Brown et al., 2004a; Brown et al., 2004b; Brown et al., 2006). Consistently, studies in non-medicated schizophrenic patients have demonstrated a

decrease in overall NGF expression, which has been suggested to account for neurodevelopmental abnormalities (Aloe, Iannitelli, Angelucci, Bersani, & Fiore, 2000; Parikh, Evans, Kahn, & Mahadik, 2003).

Sixth, the polymerase chain reaction (PCR) analyses from our laboratory have shown a significant decrease of alpha7 nicotinic receptor genetic expression in the hippocampus of D_2 primed animals. Consistently, studies have shown decreases in density of the alpha4beta2 nicotinic receptor in the hippocampus, decreases in the alpha7 nicotinic receptor subunit gene, as well as a reduction in muscarinic receptor availability in vivo in human schizophrenic patients (Durany et al., 2001; Leonard et al., 2002; Raedler et al., 2003).

Finally, neonatal quinpirole treatment in rats produced significant decreases in the regulator of G-protein signaling (RGS) 9, the genetic transcript that codes for the G-protein that couples to the D_2 receptor, when D_2 primed animals are adults. This has also been shown to be consistent with the microarray experiments in postmortem schizophrenic patients (Mirnics, Middleton, Stanwood, Lewis, & Levitt, 2001). Mirnics et al. found that RGS 4 expression was significantly decreased in the prefrontal cortex of the schizophrenic patients. Therefore, neurochemically and behaviorally, it can be concluded that the dopamine D_2 priming receptor model is the most consistent with symptoms of schizophrenia in humans.

Conditioned Place Preference

The conditioned place preference (CPP) is a paradigm that was developed to measure the associative effects of drugs and distinctive environmental cues (Carr, Fibiger, & Phillips, 1981). This relationship has been developed in rodents (Bienkowski, Kuca,

Piasecki, & Kostowski, 1996). The CPP behavioral apparatus usually consists of either two or three chambers. For the two-chambered CPP apparatus, usually one chamber is painted black, and the other chamber is painted white. In the three-chambered CPP apparatus, the chambers are generally painted black, white, and gray. Regardless of the apparatus used, each chamber has a different texture so that rodents can discriminate between the chambers with tactile stimuli as well as through the different color of each chamber.

Pavlov (1927) explained that classical conditioning involves a conditioned stimulus (CS) and an unconditioned stimulus (UCS) and defined reinforcement as strong association between the CS and UCS. The CPP is based on classical conditioning principles and involves a temporal pairing of a CS, a particular compartment, and a UCS, the drug, to measure reinforcement. In the drug-induced CPP, the conditioned response (CR) and unconditioned response (UCR) are the amount of time the animals stay in each chamber or the amount of locomotor activity that the animals exhibit after the CS is exposed. Subjects' CR and UCR are measured in order to understand drug reinforcement.

The CPP procedure requires repeated pairings of a UCS and CS. This repeated pairing has been shown to develop a CR in animals (Carew & Rudy, 1991; Shippenberg & Heidbreder, 1995). After conditioning, when allowed to freely access all chambers after removing the dividers of the CPP apparatus, animals usually prefer the environment paired with the drug. This preference is the indicator of drug-induced CPP in animals. Also, developed CS and CR relationships have been shown to block animals' ability to become habituated to a new CS (Carew & Rudy). Although CPP was developed to test the associative effects of drugs, it has also been reported to be a useful device for

measuring the degree to which drugs alleviate anxiety (anxiolytic effect) (Matsuzawa, Suzuki, & Misawa, 2000; Torrella, Badanich, Philpot, Kirstein, & Wecker, 2004).

In general, the CPP procedure usually consists of three major steps: pretest, conditioning, and posttest. The pretest is an initial test conducted on animals without any pre-drug exposure to the apparatus. During the pretest, animals are individually placed in the center of the shuttle box, and their initial compartment preference is obtained by measuring the amount of time they spend in each compartment. During the conditioning phase, one compartment is repeatedly paired with a psychoactive drug and the other compartment is paired with saline. The posttest is typically conducted the day after the final conditioning trial to record the strength of conditioning and which compartment is preferred by the animal. Therefore, the CPP is used to measure associative and reinforcing effects of positively reinforcing drugs, using classical conditioning principles.

Biased and Unbiased Designs

There are two different designs in the CPP: biased and unbiased (R. W. Brown, personal communication, February 26, 2007). The biased design allows animals to freely access any of the compartments to see which compartment they prefer when they are first exposed to the apparatus. Then, the animals are conditioned to the initially non-preferred compartment that is paired with a drug. In contrast, in the unbiased design, the animals' initial chamber preference is not taken into account for chamber assignment, so half of the animals are randomly assigned to one compartment and the rest of the animals to another compartment. In this case, both compartments are paired with reinforcing drugs (R. W. Brown, personal communication).

Even though both CPP designs have been shown to develop several psychostimulant drug-induced CPP, it has been claimed that the biased design should be used over the unbiased to measure the reinforcing effect of nicotine (Le Foll & Goldberg, 2005). Le Foll and Goldberg analyzed which nicotine dose and which CPP design (biased or unbiased) would produce nicotine-induced CPP in rats. They found that when rats are injected with 0.1 to 1.4 mg/kg of nicotine and placed in their non-preferred compartment (biased design), nicotine induced CPP. Also, they reported nicotine induced conditioned place aversion when rats were given 2.0 mg/kg of nicotine and initially placed in their preferred compartment (Le Foll & Goldberg). A possible explanation is that the biased design is able to detect differences between before versus after conditioning because rats are initially placed in the non-preferred chamber (Schenk, Ellison, Hunt, & Amit, 1985; Scoles & Siegel, 1986).

The Two- Versus Three-Chambered CPP

In general, most CPP used studies use either the two- or the three-chambered CPP apparatus. In both paradigms, reinforcing drugs including amphetamine, apomorphine, morphine, cocaine, and nicotine have been shown to induce CPP (Belluzzi et al., 2004; Brielmaier, McDonald, & Smith, 2007; Le Foll & Goldberg, 2005; Nazarian, Russo, Festa, Kraish, & Quinones-Jenab, 2004; Parker, 1992; Vastola, Douglas, Varlinskaya, & Spear, 2002). However, Torrella et al. (2004) reported that the use of the two-chambered CPP apparatus may produce confounding results. They concluded that obtained results from the two-chambered CPP could reflect either animal's preference for a particular chamber or the animals' aversion to another chamber. This aversion to a particular chamber means that they avoid the other chamber rather than prefer the chamber they are

in. Therefore, it is difficult to determine whether the animal's preference for a particular chamber is due to the reinforcing effect of the drugs or the anxiolytic effect of the drugs because CPP can measure the anxiolytic effect of the drugs when it follows the biased design (Davis, Roma, Dominguez, & Riley, 2007; Torrella et al.).

Torrella et al. (2004) conducted a three-chambered CPP study with nicotine in adolescent and adult rats to see if the two-chambered CPP may be measuring the aversion to the other compartment. They reported that after conditioning sessions with nicotine, the anxiolytic effect of nicotine was observed in P 39 rats but not in P 69 rats (Torrella et al.). In addition, Vastola et al. (2002) showed that nicotine-induced CPP was observed in adolescents but not in adults when they used the two-chambered CPP apparatus and followed a similar procedure as Torrella and colleagues' study. In contrast, it has been reported that nicotine does not induce CPP in adolescent rats when using the three-chambered CPP apparatus but does in the two-chambered CPP apparatus (A. Rauhut, personal communication, June 12, 2006). Therefore, others have reported that the two-chambered CPP apparatus may be preferred when testing nicotine-induced CPP in adolescent rats (A. Rauhut, personal communication; Torrella et al.).

In conclusion, nicotine-induced CPP is an elusive and difficult finding because it can be easily affected by age and the apparatus. Even though nicotine is a reinforcing drug, reinforcements from nicotine are not as strong as from such highly addictive drugs as morphine and cocaine. In general, the features of the two- and the three-chambered CPP apparatuses are very similar because they contain either black/white boxes or black/gray/white boxes. However, as Torrella et al. (2004) explain, the three-chambered CPP apparatus may be somewhat more sensitive in detecting nicotine-induced CPP than

the two-chambered CPP. Therefore, it can be concluded that the three-chambered CPP apparatus is a better way to assess nicotine-induced CPP.

In addition to Torrella and colleagues' claim, there are a few additional advantages reported to using the three-chambered CPP apparatus rather than the two-chambered CPP apparatus. First, Tzschentke (1998) reported that having a third compartment provides a neutral environment that can serve as a starting point. As a result, the three-chamber CPP paradigm allows the subject to decide which chamber it prefers (Torrella et al., 2004).

Secondly, Parker (1992) performed a study using the three-chambered CPP paradigm in rats to see if outcomes of the CPP are due to novelty interference with habituation. Several psychoactive drugs were paired (amphetamine, apomorphine, and morphine) to one compartment, and saline was paired to the other compartment. The results showed that rats preferred a compartment paired with psychoactive drugs more than the other compartments. Interestingly, the results also showed that rats repeatedly preferred a novel chamber more than a compartment paired with saline (Parker). Therefore, it appears that the three-chambered CPP has the advantage of measuring outcomes in the CPP on the drug-free test that the two-chambered CPP apparatus presumably cannot measure (Tzschentke, 1998). However, there is not much literature that discusses the two- versus three-chambered CPP issue, so further research on this topic is required.

Adolescent Animals and the CPP

Several studies have shown that nicotine only induced CPP consistently in adolescent animals but not in adults. Brielmaier et al. (2007) used the two-chambered

CPP paradigm and biased design in early adolescent and adult rats. They found that acute injection of nicotine induced CPP in early adolescent animals but not in adult rats. Furthermore, Belluzzi et al. (2004) have shown that early adolescent rats (P 28) showed nicotine-induced CPP when they are given acute nicotine administration of 0.5 mg/kg but not late adolescent (P 38) or adult rats (P 90). In addition, when late adolescent and adult rats were given nicotine repeatedly, late adolescent rats exhibited more tolerance and sensitization to nicotine than adult rats. Both studies concluded that adolescents are more sensitive to the reinforcing effect of nicotine, especially early adolescent animals (Belluzzi et al.; Brielmaier et al.)

Furthermore, Vastola et al. (2002) found that even relatively lower doses of nicotine administration induced CPP in adolescent rats but not in adult rats when they used the two-chambered CPP apparatus and biased design. They hypothesized that this is because adolescents are more likely to be nicotine dependent and sensitive to nicotine than adults, so adolescent rats showed nicotine-induced CPP even with relatively lower doses of nicotine (0.6 mg/kg) (Vastola et al.). Furthermore, neuroanatomically, it has been reported that adolescent and adult rats differ. Kalsbeek, Voorn, Buijs, Pool, and Uylings (1988) found that rats' dopamine containing fibers keep developing until rats are 60 days old. These results appear to show that only adolescent rats may demonstrate nicotine-induced CPP.

To sum up these studies, it can be concluded that the biased design should be used to measure nicotine-induced CPP and the dose should be in the range of 0.1 to 1.4 mg/kg (Belluzzi et al., 2004; Brielmaier et al., 2007; Le Foll & Goldberg, 2005; Vastola et al., 2002). Also, it is important to note that adolescents should be used to measure nicotine-

induced CPP because adults are less likely to exhibit nicotine-induced CPP. In other words, nicotine-induced CPP appears to be very age specific (Belluzzi et al.; Vastola et al.).

Sex Differences and the CPP

Several studies have reported that there may be sex differences in psychostimulant-induced CPP (Balda, Anderson, & Itzhak, 2006; Nazarian, Russo, Festa, Kraish, & Quinones-Jenab, 2004; Russo et al., 2003a; Russo et al, 2003b). Balda et al. showed that even though cocaine-induced CPP appears in both female and male adult rats, females exhibited cocaine-induced CPP to a stronger degree. Females preferred the compartment paired with cocaine more than males. This is thought to be because females are more sensitive to cocaine due to hormonal effects (Balda et al.).

Other studies have supported this hypothesis, and also that male rats take more sessions to show cocaine-induced CPP (Russo et al. 2003a; Russo et al. 2003b). To determine whether this result is due to hormonal differences, Russo et al. (2003a) gonadectomized both male and female rats. This gonadectomy procedure did not influence cocaine-induced CPP in males or females. However, ovariectomized female rats spent less time in the cocaine paired chamber than non-ovariectomized female rats, although cocaine-induced CPP was observed in both groups of female rats. Russo et al. (2003a) concluded that these sex differences in cocaine-induced CPP may be due to decreased levels of dopamine and 5-HT produced by ovariectomy. Furthermore, Nazarian et al. (2004) found that cocaine-induced CPP was observed in female rats and reasoned that this sex difference in cocaine-induced CPP resulted from different sensitivities to cocaine in the dopamine D_1 receptor. In conclusion, these studies suggest that sex

differences in D_2 primed adolescent rats may be observed in nicotine-induced CPP. However, there are not any reports of sex differences observed in nicotine-induced CPP in current published literature. If sex differences are observed in nicotine-induced CPP, it would mean that nicotine-induced CPP may be mediated by hormonal effects. Also, dopamine and 5-HT may be involved in nicotine-induced CPP differently in males and females.

Stress, the Elevated T-Maze, and the CPP

Some studies have reported that in patients with schizophrenia, dopamine and stress levels are increased, as stress and dopamine levels are reported to be positively correlated (Cabib & Puglisi-Allegra, 1996; Carlsson & Lindqvist, 1963; Finlay & Zigmond, 1997; Yilmaz et al., 2007). In addition, Rodgers, Nikulina, and Cole (1994) reported that when mice were treated with 0.5 mg/kg of a D_2 receptor agonist, they experienced increased stress and anxiety responses. On the other hand, treatment with 2.5-20.0 mg/kg of a D_2 receptor antagonist decreased those responses (Rodgers et al.). Therefore, there is a possibility that the dopamine D_2 receptor priming model of schizophrenia, which is produced by neonatal quinpirole treatment, increases stress and anxiety levels.

The increased stress and anxiety levels could possibly influence the result of the CPP. When rats are experiencing increased stress and anxiety levels, their preference for the darker colored environment increases because rats are nocturnal. Because the CPP apparatus contains black and white chambers, increased stress and anxiety levels could drastically differentiate the results among D_2 and non-D_2 primed animals. Therefore, it is

important to determine whether D_2 primed animals have higher stress and anxiety responses than non-D_2 primed animals.

The elevated T-maze (ETM) was developed to test the animal model of anxiety and fear (Graeff, Viana, & Tomaz, 1993). It tests fear and stress responses in four consecutive trials: baseline, avoidance 1, avoidance 2, and escape (Trivedi & Coover, 2004). Because this experiment is conducted in 1 day, the ETM measures the acute stress and fear responses of rodents, rather than chronic stress and fear levels. The ETM has three arms: one enclosed arm and two open arms (Zangrossi & Graeff, 1997). The open arms are located facing each other, and the enclosed arm is located perpendicular to the two open arms (Graeff, Netto, & Zangrossi, 1998; Zangrossi & Graeff). The ETM is capable of measuring two different types of fear or anxiety in animals: conditioned and unconditioned fear (Graeff et al., 1998; Viana, Tomaz, & Graeff, 1994). Conditioned fear is determined in the first three of the four consecutive trials based on how long the animal takes to leave the enclosed arm to enter either of the two open arms (Zangrossi & Graeff). On the fourth trial, unconditioned fear is measured. It is determined by how long the animal takes to leave the open arm to enter the enclosed arm (Zangrossi & Graeff). In conclusion, because the ETM is capable of measuring both conditioned and unconditioned fear, the ETM results of D_2 and non-D_2 primed animals can determine whether results obtained in the CPP are influenced by D_2 priming, or not.

Focus of the Study

Nicotine is a widely abused psychostimulant drug, especially in adolescents. Also, clinical studies have shown that schizophrenic patients are two to five times more likely to abuse psychoactive drugs, especially nicotine (de Leon et al., 1995; Hughes et al.,

1986; LeDuc & Mittleman, 1995; O'Farrell et al., 1983). Therefore, this study will use the D_2 receptor priming model of schizophrenia to analyze nicotine-induced CPP in both early postweanling (P 22-P 40) and adolescent (P 29-P 47) rats.

Adolescents demonstrate a significantly different response to nicotine than adults on several behavioral and neurochemical measures. A report indicated that around 42 % of teenagers have some experience with smoking cigarettes (U.S. Department of Health and Human Services, 1991 as cited in Wang et al., 1999). The initiation of smoking in adolescence leads to a lesser likelihood of termination of smoking in adulthood (DiFranza et al., 2000; Smith et al., 2006). Furthermore, rodent studies have shown that adolescents have higher sensitivity to nicotine than adults (Belluzzi et al., 2004; Brielmaier et al., 2007; Rezvani & Levin, 2004). Therefore, it is not surprising that there are age differences in the neurochemical and behavioral responses to nicotine in adolescence compared to adulthood (Abreu-Villaca et al., 2003; Belluzzi et al.; Brielmaier et al.; Trauth et al., 2001). However, there is no defined mechanism as to why this age difference in drug effects exists.

The CPP is an excellent measure of the associative effects of drugs and environmental context (Bienkowski et al., 1996; Carr et al., 1981). There are several advantages to using CPP to study drug addiction. First, it is easily applicable to situations by measuring how environmental cues can trigger relapse. Second, it measures behavioral outcomes when the drug is not present (Tzschentke, 1998). Third, CPP has been reported to be effective for testing the anxiolytic or anxiogenic effects of drugs (Torrella et al., 2004). Finally, the CPP may be especially effective at testing the effects of nicotine in adolescent rats. Findings have shown that nicotine-induced CPP can

generally be produced in adolescent rats, but this has not been the case in adults (Brielmaier et al., 2007; Vastola et al., 2002). Studies have shown that early postweanling rats (P 28) and adolescent rats (P 39) demonstrated nicotine-induced CPP, whereas adult rats (P 69 or P 90) did not demonstrate nicotine-induced CPP (Belluzzi et al., 2004; Torrella et al.).

Rodgers et al. (1994) found that quinpirole treatment produced increased stress and anxiety responses in mice on the elevated plus-maze test. Therefore, in the present study, rats neonatally treated with quinpirole may demonstrate a significant increase in stress levels on the CPP tests. In order to confirm this hypothesis, an ETM behavioral task was added as Experiment 2b to the behavioral tasks used in the present study. The ETM is a task that has been used to test behavioral stress in animals (Graeff et al., 1993). In Experiment 2b, a separate group of adult rats at P 60, which were neonatally treated with either quinpirole or saline, was tested on the ETM.

The aim of this study was to investigate the interaction of nicotine with different ages of adolescent D_2 primed rats using both the two- and three-chambered CPP apparatuses. Essentially, the experimental design for these CPP experiments was the same, with the only difference between the two being the different apparatuses utilized.

Hypotheses

Hypothesis 1. Neonatal quinpirole treatment (D_2 priming) will produce higher stress and anxiety levels in rats; therefore, D_2 primed animals will prefer the darker colored compartment. This hypothesis is based on the following findings. First, a microdialysis study in rats has shown that when mild stressors were present, dopamine and norepinephrine levels in the medial frontal cortex significantly increased (Cenci,

Kalen, Mandel, & Bjorklund, 1992). In addition, an elevated plus-maze study by Rodgers et al. (1994) reported that mice injected with 0.5 mg/kg of quinpirole demonstrated an increase in freezing behavior and showed less exploratory behaviors. Furthermore, rats are nocturnal, so they naturally prefer a darker colored environment, especially when they are stressed and have a high anxiety level.

Hypothesis 2. Animals that are conditioned with nicotine will show nicotine-induced CPP regardless of neonatal treatment. This is based on findings that have shown nicotine induced CPP in adolescent rats (Janhunen, Linnervuo, Svensk, & Ahtee, 2005; Le Foll & Goldberg, 2005).

Hypothesis 3. Animals neonatally treated with quinpirole and conditioned with nicotine will produce a higher preference for the white compartment compared to animals neonatally treated with saline and conditioned with nicotine. The basis for this hypothesis is that neonatal quinpirole treatment produces a rodent model of schizophrenia, and approximately 85 % of the schizophrenic population in America is nicotine dependent (de Leon et al., 1995; Hughes et al., 1986; O'Farrell et al., 1983). Also, D_2 priming increases dopamine activity that reinforces the effects of nicotine.

Hypothesis 4. Nicotine will enhance CPP in younger animals due to possible withdrawal effects from chronic quinpirole treatments. This hypothesis is based on observations from past studies. Adult rats tend to become sick after nicotine injections and take longer to build tolerance to nicotine than younger animals. The possible reason for this age difference may be because adult rats experience withdrawal effects from chronic quinpirole treatments more severely than younger animals.

Hypothesis 5. Animals neonatally treated with quinpirole will demonstrate a significant increase in behavioral stress on the ETM. This increase in stress will be manifested as a significant increase in freezing behavior, which is the species-specific response for fear in rats, on the escape trial of the ETM. This hypothesis is based on the finding showing that mice treated with quinpirole demonstrated increased stress response on the elevated plus-maze (Rodgers et al., 1994).

CHAPTER 2

METHODS

Experiment 1: The Two-Chambered CPP

Subjects

In this study, two age groups were used: early postweanling (P 22-P 40) and adolescent (P 29-P 47). Female and male Sprague-Dawley (SD) rats were obtained from Harlan Laboratories, Inc (Indianapolis, ID) and mated in order to produce offspring that were used as subjects. A total of 67 SD male and female rats (early postweanling group n = 36; adolescent group n = 31) were used for Experiment 1. After neonatal drug treatment was complete, all animals were weaned at P 21 and assigned to their respective groups. All of the animals were assigned to either saline or nicotine (experimental group: Male Saline Nicotine, Male Quinpirole Nicotine, Female Saline Nicotine, and Female Quinpirole Nicotine; control group: Male Quinpirole Saline, Male Saline Saline, Female Quinpirole Saline, and Female Saline Saline). The animals were socially housed in cages in groups of two to three in a climate-controlled vivarium with food and water available ad libitum with a 12 hour on/off light/dark cycle. All procedures are approved by the University Committee on Animal Care (UCAC) at East Tennessee State University (ETSU) and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Apparatus

The two-chambered CPP paradigm was constructed of plywood (30 inch long x 18 inch wide x 20 inch high) and consisted of a black colored chamber and a white colored chamber. These chambers were separated by a removable painted wooden

divider. The walls of the chambers were painted with a flat finish. The bottom of the white colored chamber was painted flat white and covered with studded plastic. The bottom of the black colored chamber was painted with a textured black paint. The different textures were used so that the animals could better discriminate between the black and white chambers. The apparatus was located in a room surrounded with several electric fans, which helped to eliminate background noise. A video camera was located on the ceiling approximately 10 feet above the CPP chamber for recording purposes. The overhead lights were turned on during the conditioning sessions and testing trials.

Procedure

Neonatal Injection. All of the animals were injected intraperitoneally (i.p.) with either quinpirole HCl (1 mg/kg) or saline (0.9 %, 1 mg/kg) from P 1 to P 21. The animals were injected at approximately 9:00 AM every day.

Early postweanling/Adolescent Injection. On pretest, posttest1, and posttest 2 all of the animals were injected i.p. with saline only (0.9 %, 1 mg/kg). During the mornings of conditioning days (approximately 9:00 AM daily), all of the animals were i.p. injected with the drug they were assigned, which was either nicotine (0.8 mg/kg free base) or saline (0.9 %, 1 mg/kg), 10 minutes prior to being placed into the CPP apparatus. In the afternoons (approximately 1:00 PM daily), all of the animals received only saline (0.9 %, 1 mg/kg), 10 minutes prior to being placed into the CPP apparatus.

Pretest. Pretests were given at two different ages. For the early postweanling rats, the pretest was administered 1 day after weaning at P 22. In the adolescent rats, the pretest was given at P 29. All animals were given a pretest trial to determine initial preference. The dividers between each chamber were removed before the pretest, in order

to allow the animals to have free access to all chambers in the apparatus. Each animal was released on the dividing line of the black and white chambers in the two-chambered CPP apparatus. Animals were left in the box for 5 minutes (300 seconds) and the video camera recorded all animals' movements on videotape, which was later scored by the experimenter. For each subject, the amount of time spent in each chamber was recorded. Additionally, the number of shuttles made by each animal, which were defined as the movement from one chamber to the other, was also recorded.

Conditioning. The early postweanling rats were conditioned from P 23 to P 39, while adolescent rats were conditioned from P 30 to P 46. Conditioning sessions began the day after the pretest. For all conditioning trials, the removable wooden dividers were placed back into each chamber. All animals were given two conditioning sessions (morning and afternoon) per day. In the morning session (started at approximately 9:00 AM daily), the animals injected with nicotine were placed in the white compartment 10 minutes after the injection and spent 300 seconds in the chamber. Animals injected with saline were placed into the black chamber. In the afternoon session (started at approximately 1:00 PM daily), animals that were injected with saline in the morning and were also injected with saline in the afternoon session were placed in the white chamber. Rats that were injected with nicotine in the morning and saline in the afternoon were placed in the black chamber. After each conditioning session, all of the animals were placed back in their home cage. All animals were given a total of 16 days of conditioning. There was a posttest given after the first 8 conditioning days, and another posttest given after 16 days of conditioning. This procedure is based on that of Torrella et al. (2004), which showed that this procedure produced nicotine-induced CPP in adolescent rats.

Posttests. There were two posttests; posttest 1 was administered in early postweanling rats at P 31, and posttest 2 at P 40. Adolescent rats were given posttest 1 at P 38 and posttest 2 at P 47. On each posttest, all of the animals were given saline. The dividers between the chambers were removed in order to let the animals freely explore the CPP apparatus. The animals were released into the center of the CPP box and left in the box for 300 seconds. The video camera recorded movement, and the experimenter later recorded the amount of time each rat spent in each chamber as well as the number of shuttles. After the sessions were administered and recorded, the animals were placed back in the cages and returned to the animal colony.

Experimental Design. An initial 2 (age: early postweanling or adolescence) x 2 (neonatal drug treatment: quinpirole or saline) x 2 (early postweanling/adolescent drug treatment: nicotine or saline) x 2 (repeated measure: posttest 1 or 2) four-way analysis of variance (ANOVA) was performed and a robust significant age main effect was revealed $F(1, 59) = 45.88$, $p < .001$. Based on this significant age difference, it was decided to analyze the two age groups separately. For each age, A 2 (neonatal drug treatment) x 2 (early postweanling/adolescent drug treatment) x 2 (repeated measure) three-way ANOVA was used. The dependent measure, which was calculated in seconds, was the amount of time spent in each chamber. It was expressed as the amount of time spent in the white chamber subtracted from the amount of time spent in the black chamber.

Experiment 2

Subjects

Experiment 2a: The Three-Chambered CPP. Like in Experiment 1, two age groups were used: early postweanling and adolescent. A total of 62 SD male and female

rats (early postweanling group n = 36; adolescent group n = 26) were used. All of the animals were assigned to either saline or nicotine (experimental group: Male Saline Nicotine, Male Quinpirole Nicotine, Female Saline Nicotine, and Female Quinpirole Nicotine; control group: Male Quinpirole Saline, Male Saline Saline, Female Quinpirole Saline, and Female Saline Saline). After neonatal drug treatment was complete, all animals were weaned at P 21 and assigned to their respective groups.

Experiment 2b: The ETM. In this study, adult rats (P 60) were used as subjects. A total of 38 SD adult male and female rats (Female Quinpirole Saline group n = 7; Female Saline Saline group n = 10; Male Quinpirole Saline group n = 9; Male Saline Saline group n = 12) were used. All of the animals were assigned to saline only (experimental group: Female Quinpirole Saline and Male Quinpirole Saline; control group: Male Saline Saline Female Saline Saline). After neonatal drug treatment was complete, all animals were weaned at P 21 and assigned to their respective groups.

The animals were socially housed in cages in groups of two to three in a climate-controlled vivarium with food and water available ad libitum with a 12 hour on/off light/dark cycle. All procedures are approved by the UCAC at ETSU and the vivarium is fully accredited by the AAALAC.

Apparatus

Experiment 2a. The three-chambered CPP apparatus was constructed of plywood (30 inch long x 12 inch wide x 24 inch high) and consisted of a black colored chamber on one end, a white colored chamber on the other end, and a gray colored chamber in between the black and white chambers. All of the chambers were separated by a removable painted wooden divider. The walls of the black, white, and gray chambers and

the bottom of the gray chamber were painted with a flat finish. The bottom of the white colored chamber was painted a glossy white, and the bottom of the black colored chamber was painted with a textured black paint to help the animals discriminate between the black and white chambers. The three-chambered CPP apparatus was in the same room as the two-chambered CPP apparatus and was also surrounded by electric fans that helped to eliminate background noise. A video camera was located on the ceiling approximately 10 feet above the three-chambered CPP paradigm. The overhead lights were turned on during the conditioning sessions and testing trials.

Experiment 2b. The ETM was constructed of wood and Plexiglas. It consisted of three arms (20 inch long x 5 inch wide): one enclosed arm and two open arms, which were painted black. One arm (enclosed arm) was enclosed by a 16 inch tall piece of Plexiglas. The other two arms (open arms) were enclosed by 0.4 inch tall pieces of Plexiglas for fall prevention. The whole ETM was elevated 20 inches above the floor. An experimenter was present to record the results. The overhead lights were turned off during the testing trials, and the only illumination was a dim light (75 watts).

Procedure

Experiment 2a. All of the early postweanling and adolescent animals used in Experiment 2a were given neonatal injections, early postweanling/adolescent injections, conditioning, pretest, and posttests, identical to the procedures of Experiment 1.

Experiment 2b. There were a total of four consecutive ETM trials, and all testing was administered at P 60. In the first three trials, each animal was released facing towards the center of the two open arms at the end of the enclosed arm. Each animal was given 300 seconds, and the experimenter recorded the amount of time the animal took to fully

exit the enclosed arm with all four paws. If an animal did not leave the enclosed arm within 300 seconds, it was removed from the ETM by the experimenter, and the session was ended. On the fourth trial, the animal was released at the end of the right open arm facing towards the center of the maze and given 300 seconds. The experimenter recorded the amount of time the animals took to fully exit the open arm with all four paws. After 300 seconds, the session was ended. After the sessions were administered and recorded, the animals were placed back in the cages and returned to the animal colony. This procedure is based on that of Trivedi and Coover (2004).

Experimental Design

Experiment 2a. Experimental design used in the three-chambered CPP study was identical to Experiment 1.

Experiment 2b. A 2 (gender: female or male) x 2 (neonatal drug treatment: quinpirole or saline) x 4 (repeated measure: baseline, avoidance 1, 2, and escape) three-way ANOVA was used. The dependent measure is the amount of time animals take to fully leave either the open arm or the enclosed arm, which is expressed in seconds.

CHAPTER 3

RESULTS

Experiment 1

Early Postweanling Group

The results for the early postweanling group are presented in Figure 1. A 2 x 2 x 2 three-way ANOVA revealed a significant main effect of neonatal drug treatment $F(1, 31) = 4.53$, $p < .04$ and early postweanling drug treatment $F(1, 31) = 9.05$, $p < .005$. Animals neonatally treated with saline and conditioned with nicotine (S-N Group) visited the white compartment more often than controls at posttest 1, as a lower mean on the figure indicates a stronger preference for the white chamber than the other groups. At posttest 2, both S-N and Q-N Groups visited the white chamber more often compared to controls (S-S and Q-S Groups). In addition, at both posttests, animals neonatally treated with quinpirole and conditioned with saline (Q-S Group) demonstrated a strong preference for the black chamber compared to controls. Interestingly, animals neonatally treated with quinpirole and conditioned with nicotine (Q-N Group) visited the white chamber more at both posttests compared to Q-S Group, although Q-N Group did not visit the white chamber as much as S-N Group at posttest 1. Thus, it appears that D_2 priming produced a black chamber preference, and nicotine may have alleviated the black compartment preference at both posttests.

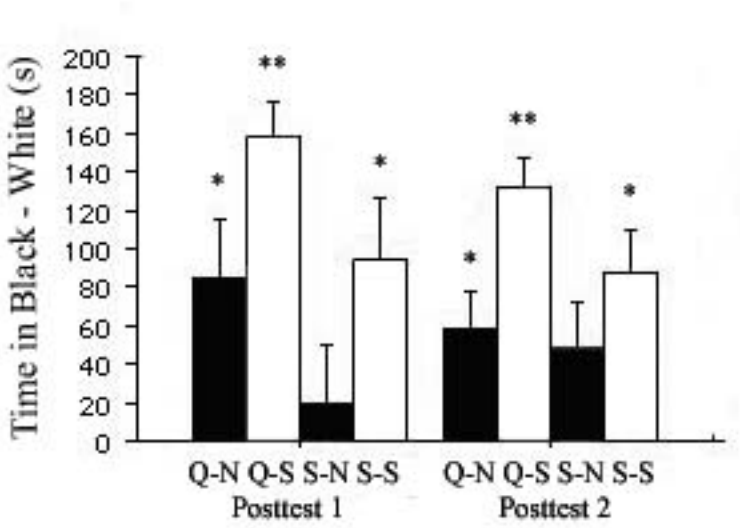

Figure 1. The Two-Chambered CPP: Early Postweanling Group.

** indicates greater than all other groups, $p < .05$. * indicates significant difference, $p < .05$.

Adolescent Group

The results for the adolescent group are presented in Figure 2. A 2 x 2 x 2 three-way ANOVA revealed a significant main effect of adolescent drug treatment $F(1, 27) = 10.55$, $p < .001$, and significant interaction of Adolescent Drug Treatment x Posttest Day $F(1, 27) = 5.26$, $p < .03$. Unlike the results from the early postweanling groups in the two-chambered CPP study, there was no significant difference between Q-N and S-N Groups at posttest 1. Both Q-N and S-N Groups visited the white compartment more often compared to Q-S Group and S-S Group, which was the group neonatally treated and conditioned with saline. Also, at posttest 2, there were no significant differences between the groups, and all groups demonstrated a significant preference for the white chamber. Thus, adolescent Q-S Group did not demonstrate a preference for the black

chamber after an additional 8 days of conditioning, as they did at a younger age in the two-chambered CPP study.

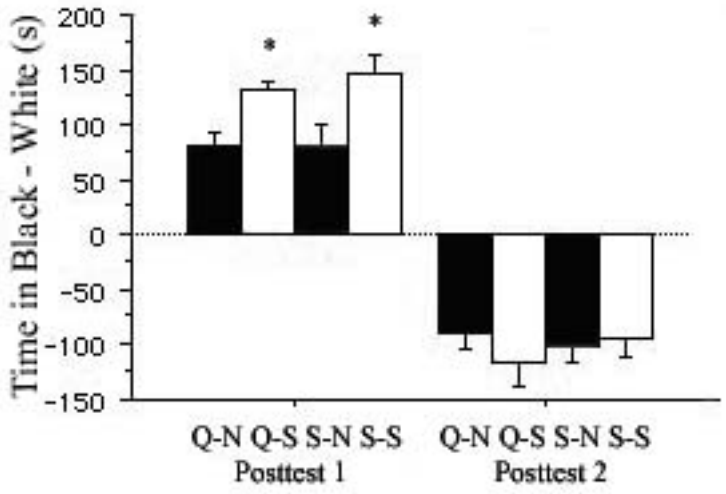

Figure 2. The Two-Chambered CPP: Adolescent Group.

* indicates significant difference, $p < .05$.

Experiment 2a

Early Postweanling Group

The results for the early postweanling group are presented in Figure 3. A 2 x 2 x 2 three-way ANOVA revealed a significant main effect of early postweanling drug treatment $F(1, 32) = 9.1$, $p < .005$, day of testing $F(1, 32) = 7.75$, $p < .009$, and significant two-way interactions of Neonatal Drug Treatment x Day of Testing $F(1, 32) = 3.95$, $p < .05$ and Early Postweanling Drug Treatment x Day of Testing $F(1, 32) = 7.30$, $p < .01$. Although Q-N Group did not equal S-N Group, both Q-N and S-N Groups visited the white chamber more often compared to Q-S and S-S Groups at posttest 1. However, at posttest 2, this nicotine-induced CPP in S-N and Q-N Groups diminished. In fact, all of animals showed a preference for the black compartment at posttest 2. Furthermore, at

both posttests, Q-S Group showed a preference for the black chamber, as younger animals had shown in Experiment 1, and nicotine appears to have alleviated this preference.

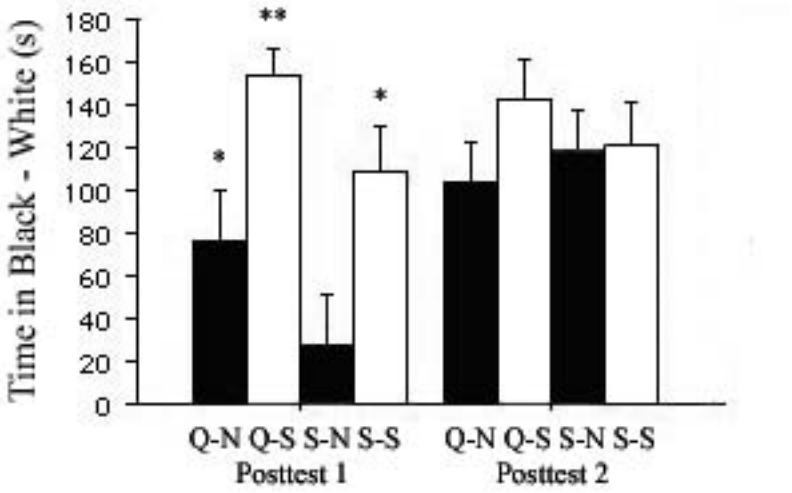

Figure 3. The Three-Chambered CPP: Early Postweanling Group.

** indicates greater than all other groups, $p < .05$. * indicates significant difference, $p < .05$.

Adolescent Group

The results for the adolescent group are presented in Figure 4. A 2 x 2 x 2 three-way ANOVA revealed a significant main effect of neonatal drug treatment $F(1, 26) = 4.36$, $p < .05$ and adolescent drug treatment $F(1, 26) = 6.63$, $p < .02$. Similar to the results observed in early postweanling groups in the three-chambered CPP study, Q-N and S-N Groups visited the white chamber more than Q-S and S-S Groups at posttest 1. In contrast, at posttest 2, this effect diminished, and there were no significant differences across groups. In addition, the black compartment preference observed in Q-S Group at

posttest 1 was not persistent at posttest 2. It appears that nicotine alleviation of the black compartment preference in D_2 primed animals was not persistent to posttest 2.

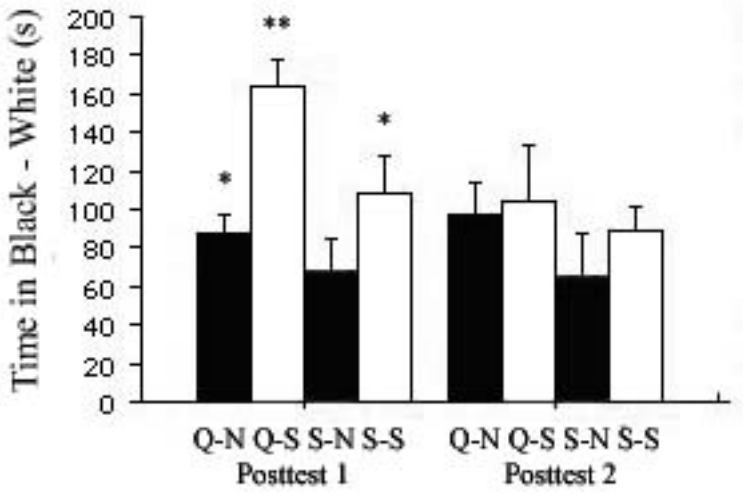

Figure 4. The Three-Chambered CPP: Adolescent Group.

** indicates greater than all other groups, $p < .05$. * indicates significant difference, $p < .05$.

Experiment 2b

Baseline and Avoidance Trials

The results of baseline and avoidance trials in both D_2 and non-D_2 primed groups are presented in Figure 5. A 2 x 2 x 4 three-way ANOVA revealed a significant main effect of gender $F(1, 34) = 5.60$, $p < .03$, but no other main effects or interactions were statistically significant. Female animals took significantly less time to enter one of the open arms than the male animals in the first three trials. There was no effect of neonatal drug treatment on baseline and avoidance trials. This result demonstrated the lack of statistical effect on conditioned fear between D2 and non-D2 primed animals.

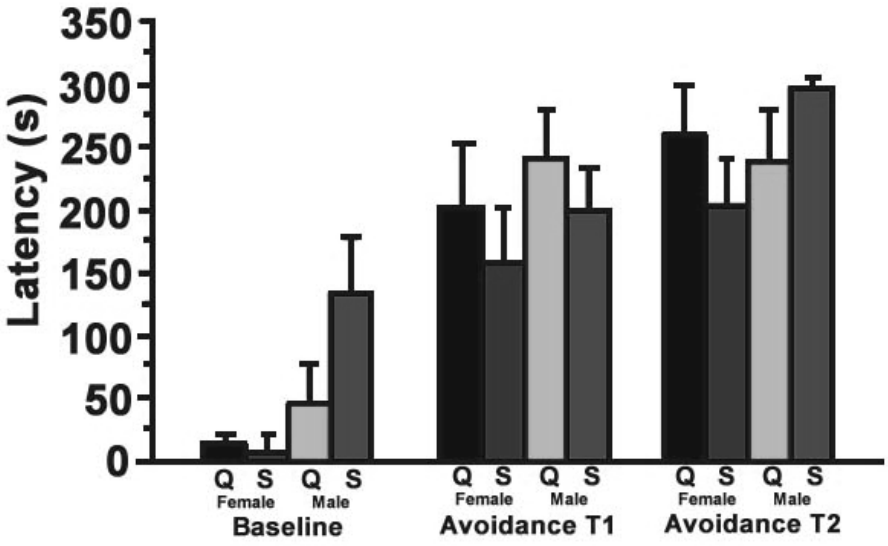

Figure 5. Elevated T-maze: Baseline and Avoidance Trials.

Escape Trial

The results of escape trials in both D_2 and non-D_2 primed groups are presented in Figure 6. A 2 x 2 ANOVA revealed a significant main effect of neonatal drug treatment, but no significant main effect of sex or significant interaction of Neonatal Drug Treatment x Sex. D_2 primed animals remained in the open arm for a longer period of time than non-D_2 primed rats. This result appears to indicate that neonatal quinpirole treatment produced significant increases in stress and possibly unconditioned fear response in D_2 primed rats.

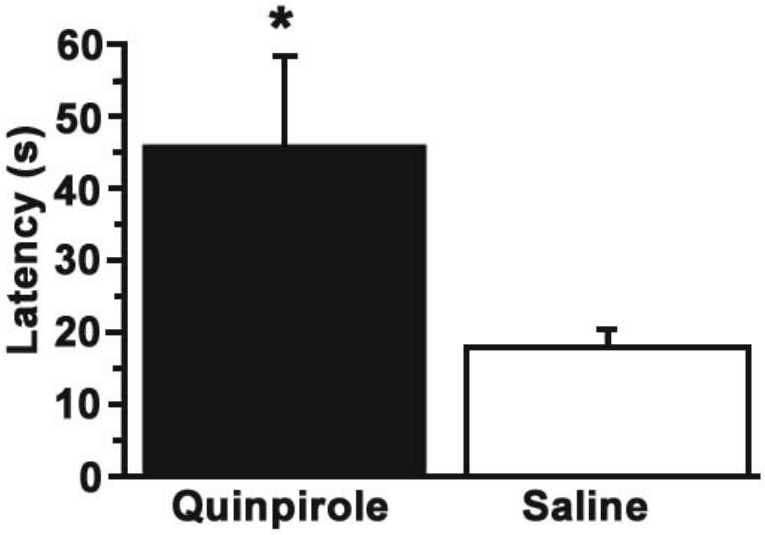

Figure 6. Elevated T-Maze: Escape Trial.

* indicates significant difference, $p < .05$.

CHAPTER 4

DISCUSSION

The aim of this study was to investigate how nicotine-induced CPP develops in both D_2 and non-D_2 primed early postweanling and adolescent animals using both the two- and three-chambered CPP paradigms. Overall, consistent results were observed in early postweanling and adolescent animals at posttest 1 (P 31 and P 38) in both studies. First, nicotine induced CPP in both early postweanling and adolescent rats neonatally treated with either saline or quinpirole. Also, neonatal quinpirole treatment produced an aversion to the white compartment and a preference for the black chamber in D_2 primed animals. Third, nicotine appeared to alleviate this preference for the black compartment. In addition to the two- and three-chambered CPP studies, D_2 and non-D_2 primed rats were behaviorally tested on the ETM to investigate whether D_2 primed rats demonstrated an increased stress response. Indeed, results showed that D_2 primed animals exhibited significantly elevated fear compared to non-D_2 primed animals on the ETM. Based on this result, D_2 priming appears to elevate stress and fear levels. This is especially true of unconditioned fear, but not conditioned fear.

There were significant age differences observed in both the two- and three-chambered CPP studies. At posttest 1 early postweanling S-N Groups visited the white compartment more than the adolescent S-N Groups. This effect may stem from different dopamine D_2 densities. Another age difference is that at posttest 2, none of the adolescent animals showed a significant preference for the black compartment, while early postweanling animals did show a preference for the black compartment. This may be due to an age-related difference in stress levels. As rats approach adulthood, there is a

reduction in the fear response to a stressful stimulus such as the white compartment. This finding also demonstrates that behavioral performance differed based on the apparatus used and the age of the animal.

Experiment 1

Early Postweanling Group

In Experiment 1, results revealed that early postweanling Q-S Group showed a significant preference for the black chamber at both posttests (P 31 and P 40). This result, combined with the results from Experiment 2b, appears to show that D_2 primed animals have increased stress levels that may produce a preference for the darker colored compartment. On the other hand, Q-N Group did not show a significant conditioned place aversion at both posttests (P 31 and P 40). This effect is hypothesized to occur because nicotine alleviated the increased anxiety and stress levels in D_2 primed animals. This hypothesis is supported by Brioni, O'Neill, Kim, and Decker (1993), who reported that nicotinic receptor agonists such as nicotine and lobeline have anxiolytic effects in rodents behaviorally tested on the elevated plus-maze. They found that mice that were injected either with nicotine or lobeline showed reductions in their stress and fear levels by spending significantly longer in the open arms of the elevated plus-maze. Interestingly, when nicotine-treated mice were injected with a nicotinic receptor antagonist, either mecamylamine or chlorisondamine, the mice exhibited an increased stress and fear response (Brioni et al.). Therefore, it appears that nicotine has anxiolytic properties that are mediated by the nicotinic receptor. In conclusion, the results obtained from the Brioni and colleagues' study and the current study showed that neonatal quinpirole treatment consistently produced a significant preference for the black compartment, and nicotine

has alleviated this preference for the black chamber at both posttests in early postweanling rats.

An additional finding was that the early postweanling S-N Group visited the white chamber more often than controls, which means nicotine induced CPP in S-N Group at posttest 1. This finding is partially consistent with the findings of Brielmaier et al. (2006) that showed that early postweanling animals (P 28) demonstrated nicotine-induced CPP when they were given a single acute injection of nicotine in the two-chambered CPP apparatus. In the current study, nicotine-induced CPP observed at posttest 1 in S-N Group remained at posttest 2, and, in fact, S-N and Q-N Groups visited the white compartment equivalently after extended conditioning. Therefore, with further conditioning in the two-chambered CPP apparatus, nicotine is apparently able to induce CPP or alleviate stress levels completely in D_2 primed early postweanling rats.

Adolescent Group

In the two-chambered CPP study, both D_2 and non-D_2 primed adolescent rats administered nicotine (Q-N and S-N Groups) visited the white compartment more than controls at posttest 1 (P 38). This finding was consistent with Vastola et al. (2004), who reported that after 12 days of conditioning, nicotine induced CPP in 40-day old adolescent animals in the two-chambered CPP apparatus. Interestingly, in the current study, all of the adolescent groups showed a preference for the white chamber at posttest 2 (P 47). In fact, D_2 primed adolescent rats actually showed an equivalent preference for the white chamber as controls did. One of the possible reasons for this finding is that all animals were habituated to the white chamber by posttest 2. Also, the D_2 primed animals may have had more of an opportunity to visit the white chamber and were not able to

avoid the white chamber easily in the two-chambered CPP apparatus because in the two-chambered CPP apparatus, rats are given only two chambers to choose from. This interesting result was not expected; however, it is assumed that extended training and increased animal age interacted in the two-chambered CPP study to produce the increased preference for the white compartment in adolescent rats at posttest 2. In summary, it appears that in adolescence, neonatal quinpirole treatment does not have a significant effect on CPP performance, at least with the two-chambered paradigm as it was used in this experiment.

Experiment 2a

Early Postweanling Group

In the three-chambered CPP study, results revealed that at posttest 1 (P 31), early postweanling rats behaved in a similar manner as they did in the two-chambered CPP apparatus. However, unlike the results from the two-chambered CPP study, at posttest 2 (P 40), nicotine-induced CPP observed at posttest 1 in Q-N and S-N Groups diminished. In fact, at posttest 2, all of the animals showed a preference for the black compartment. This is precisely the opposite effect that was found in adolescent rats using the two-chambered apparatus. Interestingly, these animals began to avoid the white chamber with extended training, whereas adolescent rats began to prefer the white chamber with extended training. As previously mentioned, this curious result was found possibly because animals had more opportunity to avoid the aversive chamber in the three-chambered CPP paradigm. Also, it could be because nicotine-treated animals developed tolerance to nicotine, or the positive reinforcing effects of the drug have diminished.

Adolescent Group

Regarding adolescent rats in the three-chambered CPP study, results at posttest 1 (P 38) were similar to the results from the two-chambered CPP study; nicotine induced CPP, and nicotine appears to have reduced the preference for the black compartment in D_2 primed rats. In contrast, at posttest 2 (P 47), the significant preference for the black compartment observed in Q-S and S-S Groups dissipated, although neither group showed a significant preference for the white compartment, as occurred in Experiment 1 using the two-chambered CPP apparatus. Furthermore, at posttest 2, nicotine-induced CPP observed at posttest 1 in Q-N and S-N Groups diminished. As previously indicated, these findings may be due to increased tolerance to nicotine and habituation to the chamber. Also, as animals approach adulthood, the aversive properties of the white chamber appear to diminish regardless of drug treatment.

Age Differences in the Two- and Three-Chambered CPP

There were significant age differences in both the two- and three-chambered CPP studies. In each of these studies, the early postweanling S-N Group showed a greater degree of CPP compared to adolescent S-N Group at posttest 1. A possible explanation is that, as previously indicated, younger rats have higher sensitivity to nicotine than older rats (Belluzzi et al., 2004; Brielmaier et al., 2007; Rezvani & Levin, 2004). Neurochemically, this increased nicotine sensitivity in younger rats may be due to brain development, especially dopamine D_2 receptor density development, which takes place during the early postweanling period. D_2 receptor density reaches maximum number and density at P 28 and begins to decrease until adulthood (O'Boyle & Waddington, 1984; Srivastava, Morency, & Mishra, 1991; Tarazi & Baldessarini, 2000). Furthermore, a

clinical PET study in smokers has shown that nicotine activates dopamine D_2 transmissions in the ventral basal ganglia that is responsible for reward and emotion (Scott et al., 2007). Therefore, nicotine increases the amount of dopamine in the brain, and dopamine binds to the increased number of D_2 receptors in early postweanling rats, which produces positive reinforcement. Thus, early postweanling animals may experience an increased positive reinforcement from nicotine than older animals, so this may help to explain why animals at this age have a tendency to show nicotine-induced CPP.

Another age difference observed in this study was that at posttest 2, none of the adolescent animals in either the two- or three-chambered CPP studies showed a significant preference for the black compartment, while early postweanling animals did. In the two-chambered CPP study, D_2 priming did not produce a significantly increased preference for the black compartment in adolescent rats at posttest 2, but it did in early postweanling rats at both posttests. In addition, in the three-chambered CPP study at posttest 2, all of the early postweanling animals showed a preference for the black compartment, but none of the adolescent animals showed a preference for either the white or black compartment. These curious results may reflect an age-related change that interacts with the type of behavioral apparatus used. One conclusion to draw would be that D_2 primed adolescent animals may demonstrate a less robust increased stress response than their younger counterparts, or they may have less of an aversion to fearful stimuli as they grow older in both the two- and three-chambered CPP apparatuses. It is important to note that nicotine-induced CPP is difficult to achieve in adult rats, and this

may be because in part adult controls do not show as strong of an aversion to the white compartment as adolescents.

Contradicting this claim were the results of the ETM that showed adult D_2 primed animals demonstrated unconditioned fear on the escape trial, although they did not demonstrate conditioned fear on the baseline and avoidance trials on the ETM. From this finding, it can be hypothesized that as rats grow older, they experience less conditioned fear than younger rats. Thus, at posttest 2, both the CPP apparatuses may have measured only conditioned fear and resulted in adolescent animals not showing a significant preference for the black compartment.

Also, baseline and avoidance trials of the ETM study can be applied to explain why all of the adolescent animals showed a preference for the white compartment in the two-chambered CPP apparatus. When adolescent rats must visit one of the two areas, as they are forced to do in the two-chambered CPP study, the white chamber may not be as aversive as when they have more choices, as in the three-chambered CPP apparatus. In other words, adolescent D_2 primed rats visit the white compartment if they have less opportunity to avoid that stimulus. It is important to note that D_2 priming typically produces an increase in activity, and if D_2 primed animals are demonstrating an increase in locomotion, it may essentially be less possible for these animals not to spend a significant amount of time in the white compartment.

The problem with the two-chambered CPP study is that D_2 primed adolescent rats actually showed an equivalent preference for the white chamber as controls in the two-chambered CPP apparatus, rather than the predicted preference for the black chamber. The claim has been made that the two-chambered CPP apparatus tests an aversion to one

chamber over another due to a lack of alternative choices. However, in this study, it appears that when animals are given chamber choices as in the three-chambered CPP apparatus, animals tend to more easily avoid the white chamber. This may be why none of the adolescent animals showed any preference in the three-chambered CPP.

Experiment 2b

Baseline and Avoidance Trials

The ETM results of Experiment 2b revealed that as opposed to the current study's hypothesis, there was no significant difference between D_2 and non-D_2 primed animals on baseline and avoidance trials that measured conditioned fear. This finding suggests that D_2 priming does not produce increased conditioned fear.

There was a significant difference observed among female and male rats. Female animals entered one of the open arms significantly more rapidly than male animals on the baseline and avoidance trials. This is possibly because female animals have been reported to have higher activity levels in the open-field test than males, which may induce females to leave the walled area more rapidly than males in the ETM task (Blizard, Lippman, & Chen, 1975). Consistent with the current ETM study, studies using the elevated plus-maze have reported that male rats did not prefer the open arms as strongly as female rats, and female rats exhibited more exploratory behaviors than males (Johnston & File, 1991; Lucion, Charchat, Pereira, & Rasia-Filho, 1996). A study by Zimmerberg and Farley (1993) further investigated these gender differences. They reported both female animals that received chemical castration in the neonatal period and female animals that received ovariectomy in the prepuberty period did not enter the open arms as often as control female rats. On the other hand, gonadectomy in the prepuberty period and chemical

castration in the neonatal period did not affect male rats' preference for the open arms (Zimmerberg & Farley). Thus, elevated gonadal hormones play an important role in increased stress and anxiety levels only in females. In contrast, Lucion et al. reported that to reduce anxiety levels in males, gonads need to be removed during the perinatal period. In conclusion, it is hypothesized that the current study's gender differences may have originated from different periods of elevation of gonadal hormones in both males and females.

Escape Trial

Results revealed that D_2 primed animals took significantly longer to enter the enclosed arm than non-D_2 primed animals on the escape trial that measured unconditioned fear. It is hypothesized that D_2 primed rats took longer because they exhibited increased freezing behavior on the escape trial, which is rats' natural response to high in anxiety and stress. Therefore, this may indicate that D_2 primed animals showed significantly higher anxiety levels than non-D_2 primed animals. This hypothesis leads to the conclusion that D_2 priming, as produced by neonatal quinpirole treatment, increases stress and fear levels that may in part account for the overall preference for the black chamber in D_2 primed animals. This finding is consistent with the studies reporting that stress hormones and dopamine levels are often positively correlated and both are elevated in schizophrenics (Cabib & Puglisi-Allegra, 1996; Carlsson & Lindqvist, 1963; Cenci et al., 1992; Finlay & Zigmond, 1997; Yilmaz et al., 2007). Additionally, Rodgers et al. (1994) reported that D_2 receptors play a crucial role in the development of stress and anxiety levels. Therefore, these findings and the present study's escape trial results support the hypothesis that D_2 primed animals have higher stress and fear levels than

non-D_2 primed animals. In conclusion, D_2 primed animals conditioned with saline, regardless of age, showed a significant conditioned place aversion in both the two- and three-chambered CPP studies.

Conditioned and Unconditioned Fear in the ETM and CPP

Experiment 2b results revealed that D_2 priming produced increased unconditioned fear but not conditioned fear on the ETM trials. This result suggests that D_2 primed rats do not exhibit fear and stress responses if they are conditioned or habituated to the environment. This hypothesis can be applied to finding observed in both the two- and three-chambered CPP studies. At posttest 2, in both the two- and three-chambered CPP apparatuses, early postweanling and adolescent Q-S Group animals and controls were not significantly different; Q-S Groups did not show a significantly increased preference for the black compartment compared to controls except in early postweanling Q-S Group in the two-chambered CPP study. In contrast, at posttest 1, all of the early postweanling and adolescent Q-S Groups showed a stronger preference for the black compartment compared to controls in both the two- and three-chambered CPP studies. Therefore, it can be hypothesized that at posttest 1, the CPP apparatuses measured rats' unconditioned fear levels. After 8 days of conditioning, D_2 primed rats may not yet have been conditioned or habituated to the environment, so Q-S Groups showed increased unconditioned fear compared to the rest of the groups that resulted in their significant preference for the black compartment. At posttest 2, on the other hand, the CPP apparatuses may have reflected rats' conditioned fear. After 16 days of conditioning sessions, D_2 primed rats were conditioned to the environment, so D_2 primed animals and control groups may have not been significantly different.

Application to the Human Schizophrenic Population

The current CPP and ETM results did not support the hypothesis that D_2 primed rats were more likely to develop nicotine-induced CPP than non-D_2 primed rats. In fact, non-D_2 primed rats showed a greater degree of nicotine-induced CPP. Also, this study showed that D_2 primed animals experienced increased anxiety and fear levels than non-D_2 primed animals, and their increased anxiety and stress levels appeared to be reduced by the effects of nicotine. Thus, a conclusion that can be drawn is that the reason why around 74 % to 92 % of the schizophrenic population in America is nicotine dependent (de Leon et al., 1995; Hughes et al., 1986; O'Farrell et al., 1983) may be because schizophrenics smoke cigarettes in order to reduce their anxiety and stress levels. At a cursory glance, a recommendation for partial treatment for schizophrenics may be to encourage them to keep smoking cigarettes because their schizophrenic symptoms are alleviated. However, smoking cigarettes produces multiple consequences, such as lung cancer, heart and blood vessel diseases, and respiratory diseases (U.S. National Library of Medicine, 2007a). Especially when smoking habits begins in adolescence, people have a lesser chance of quitting smoking later in life (DiFranza et al., 2000; Smith et al., 2006). Thus, smoking cigarettes should not be used to reduce stress and anxiety levels in adolescent schizophrenics. Currently, there are other ways to consume nicotine such as nicotine patches and nicotine gum. Although nicotine itself produces side effects such as high blood pressure, vomiting, difficulty breathing, and agitation (U.S. National Library of Medicine, 2007b), these methods of ingestion are thought to be less harmful to humans. Thus, these methods should be encouraged to the schizophrenic population rather than cigarette smoking. However, it is ideal that future research can lead to other nicotine

consumption methods that do not produce addiction to nicotine, or side effects from nicotine, or prove that other nicotinic receptor agonists can be substituted for nicotine.

Future Studies

Several interesting results were obtained from the current study. One of the most interesting findings was nicotine induced CPP and appeared to alleviate stress produced by D_2 priming. Based on these findings, several possible future studies can be proposed. Mecamylamine, a nicotinic receptor antagonist, could be used to block nicotine's ability to alleviate stress in D_2 primed animals to verify whether nicotinic receptors mediate stress alleviation. Additionally, because D_2 priming increases stress levels, a future study could remove the adrenal glands, which regulate the stress hormone corticosterone, from the D_2 primed animals to verify whether corticosterone mediates the stress response in D_2 primed rats. A third possible study could use a D_2 receptor antagonist and focus on whether injection of a D_2 receptor antagonist in D_2 primed animals reverses the effect of neonatal quinpirole treatment. Another possible study can focus on neuroanatomy. The nucleus accumbens shell has been reported to be a mediator of CPP (Spina, Fenu, Rongoni, Rivas, & Di Chiara, 2006). Therefore, a study could focus on the CPP results after destruction of dopaminergic neurons in the nucleus accumbens shell by a specific neurotoxin such as 6-hydroxydopamine to determine how crucial a role the nucleus accumbens shell plays in the CPP task.

In conclusion, the present study revealed that nicotine has effects on both D_2 and non-D_2 primed early postweanling and adolescent rats. Overall, nicotine has been shown to induce CPP in rats neonatally treated with saline or quinpirole regardless of age. Also, this study showed that D_2 priming produces a significant preference for the black

compartment due to increased stress and anxiety levels, but this effect was alleviated by nicotine treatment. Interestingly, there was a significant difference in performance based on the type of apparatus used and animal age, pointing to the importance of behavioral methodology and age when using the CPP paradigm. Furthermore, D_2 priming did not appear to drastically elevate the positive reinforcing effects of nicotine compared to saline-treated groups, indicating that D_2 priming does not elevate the associative effects of the drug in the CPP apparatus. In addition, this study showed that there is a relationship between nicotine addiction, adolescence, and schizophrenia. Further studies should be conducted related to this matter, which may help us to understand why this relationship exists.

CHAPTER 5

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