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Chronic Olanzapine Treatment Eliminates Cognitive

Deficits Produced by Neonatal Quinpirole Treatment

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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

Stephanie K. Thacker

May 2005

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Keywords: schizophrenia, quinpirole, olanzapine, dopamine, NGF, BDNF

ABSTRACT

Chronic Olanzapine Treatment Eliminates Cognitive

Deficits Produced by Neonatal Quinpirole Treatment

by

Stephanie K. Thacker

This study evaluated the effects of chronic olanzapine treatment on cognitive performance and neurochemical function in a rodent model of schizophrenia. Animals were neonatally treated with quinpirole, a dopamine D_2 receptor agonist, or saline. Quinpirole treatment produces an increase of dopamine D_2 receptor sensitivity that extends into adulthood, known as D_2 receptor priming, similar to a phenomenon that occurs in schizophrenia. These same rats were treated in adulthood for 28 days with olanzapine, an atypical antipsychotic, or saline. Dopamine D_2-primed rats demonstrated significant deficits on a cognitive task that were alleviated by olanzapine treatment. Brain tissue analysis revealed that D_2-primed animals demonstrated a significant decrease in the neurotrophins nerve growth factor (NGF) in the hippocampus and brain-derived neurotrophic factor (BDNF) in the frontal cortex. Olanzapine treatment alleviated the decrease in NGF. The results suggest that olanzapine eliminates cognitive impairment and may have neuroprotective properties in the hippocampus of D_2-primed rats.

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CHAPTER 1

INTRODUCTION

In 1911, Eugene Bleuler, a Swiss psychiatrist, developed the term schizophrenia by combining the Greek words for "split mind". This name was to imply a fragmentation of thought processes, a split between thoughts and emotions, and a withdrawal from reality (Comer, 2001, p. 423). Today, schizophrenia is a widely known mental disorder affecting approximately one percent of the population (American Psychiatric Association, 2002, p. 308). Therefore, in any given year, over two million people in the United States suffer from the disease (National Institute of Mental Health, 2001).

Schizophrenia affects all socioeconomic groups, age ranges, and is present in men and women, although there is a higher incidence of the disease in men. Schizophrenic patients have a shorter life expectancy, a higher incident of assault and violent behavior, and suffer from a high rate of psychostimulant dependence. They also experience higher rates of anxiety disorders, such as obsessive-compulsive disorder and panic disorder (American Psychiatric Association, 2002, p. 306-8).

Schizophrenic patients suffer from two classifications of symptoms: positive and negative. Positive symptoms are normal functions that appear distorted or in excess, such as delusions, disorganized speech, and hallucinations. Negative symptoms appear as a loss of normal functions, such as blunted or flat affect (unemotional), reduction in speech (alogia), and social withdrawal. Patients may also suffer from psychomotor dysfunction, which may include awkward movements, repeated grimaces, or odd gestures. In extreme form, these may be categorized as catatonia. (American Psychiatric Association, 2002, p. 299-301; Comer, 2001, p. 427-432).

One theory of the cause of schizophrenia is biochemical abnormalities in the brain. Over the past two decades researchers have examined the neurotransmitter dopamine, which plays a role in maintaining normal motor behavior, reinforcement, and cognition. Carlsson (1977) theorized that there is an increase in dopamine function in schizophrenics. This theory states that there is an increase in sensitivity of dopamine D_2 receptors, which is supported by the fact that *all* antipsychotic drugs that are effective in treating schizophrenia block D_2 receptors with some affinity (Tollefson, 1996). Therefore, an increase in dopamine D_2 receptor function may play a major role in schizophrenia.

Much research has been conducted to confirm the theory of dopamine changes in the brain in schizophrenic patients. Brain imaging devices have been used to examine binding potential changes or changes in neurotransmitters in humans. In one study, Abi-Dargham et al. (2000) measured changes in dopamine binding potential in schizophrenic patients and control participants through single-photon computerized emission tomography (SPECT). This method detects the change in concentrations of dopamine neurotransmission in the synaptic cleft. All subjects were measured at baseline on the first day of study. Participants were measured again after administration of α-MPT, a drug that produces acute depletion of the dopamine system. Results showed that dopamine receptor availability in schizophrenic patients was significantly higher after α-MPT exposure than controls. These results indicate that dopamine was more available for α-MPT to bind to in schizophrenic patients, demonstrating an overall increase of dopamine function in schizophrenics.

Neurochemical Changes in Schizophrenia

An important aspect of the behavioral deficits produced by schizophrenia is that schizophrenics demonstrate a deficit in cognitive function as compared to controls. This deficit

has been hypothesized to be manifested in attentional deficits (Durany, Munch, Michel,& Riederer, 1999; Durany, et al., 2000). Acetylcholine (ACh) is essential to cognitive functioning and attention and increases in ACh activity have been related to enhancement in cognition (Iversen, 1998; Pepeu & Giovannini, 2004). The nicotinic acetylcholine receptor (nAChR) is a cholinergic receptor that is abundantly present throughout the brain and especially high in the hippocampus, frontal cortex, and striatum. Studies have shown that the nAChR is important in cognitive function. When treated with nicotine and tested on the 8-arm radial arm maze, animals demonstrated significant improvement in working memory (Levin, Briggs, Christopher, & Rose, 1992). However, after training to near perfect performance pretreatment with mecamylamine, a nicotinic antagonist, produced cognitive deficits in these animals (Levin, Kaplan, & Boardman, 1997). Additionally, in a study using humans as subjects, Durany et al. (2000) examined tissue samples from schizophrenic patients and discovered that these patients display a decrease in the density of alpha$_4$beta$_2$ nicotinic receptors in the striatum. Adler et al. (1998) have demonstrated that nicotine may enhance attentional concentration in schizophrenics, and thus eliminate the cognitive impairment that is demonstrated in this population (Elvevag & Goldberg, 2000).

Dopamine may also play an important role in the cognitive dysfunction observed in schizophrenics. In a comprehensive review of the literature, Friedman, Temporini, and Davis (1999) examined dopaminergic interactions within the schizophrenic and animal populations. A major deficit that schizophrenic patients display upon diagnosis is a decrease or loss of cognitive abilities, but this may be due to the modulation of increases in the dopamine system on other neurotransmitter systems, such as acetylcholine. Dopaminergic hyperfunction has been shown to produce impairments on tasks regulating selective attention, long-term memory, and action planning (Blum et al., 2000; Brown, Gass, & Kostrzewa, 2002; Harvey, Scheepers, Brand, &

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Stein, 2001; Nieoullon, 2002; Schmidtke, Schorb, Windelmann, & Hohage, 1998), and deficits in ACh have produced similar types of behavioral deficits (Bushnell et al., 2000). In several recent studies, it has been demonstrated that the activation of the dopamine D_2 receptor produces a decrease in ACh release in the hippocampus, which may be extremely important in the cognitive deficits observed in schizophrenia (Day & Fibiger, 1994; Fumagalli, Santero, Gennarelli, Giorgio, & Andrea Riva, 2001; Umegaki et al., 2001).

Neurotrophic factors are compounds that promote neuronal survival growth and maintenance of neurons. Two of these neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), have been shown to enhance ACh function (Knipper et al., 1994) and play an important role in cognitive function. Nerve growth factor (NGF) has been found to play a crucial role in the neuroplasticity of predominantly cholinergic neurons in brain development. The NGF trk A and BDNF trk B receptors are located on ACh neurons in the septohippocampal pathway. Interestingly, plasma levels of NGF have been found to be decreased in first-episode psychotics as compared to normal subjects (Parikh, Evans, Khan, & Mahadik, 2003). In a well-validated rodent model of schizophrenia utilizing early hippocampal lesions, significant decreases in NGF have also been identified (Burke, Apter, Wainer, Mufson, & Kordower, 1994). However, much less work has been done to analyze NGF levels in schizophrenia as compared to BDNF.

Toyooka et al. (2002) examined levels of brain-derived neurotrophic factor (BDNF) from blood samples extracted from healthy volunteers and chronic schizophrenic patients and found a 55% decrease in BDNF expression compared to controls. These changes may be adversely related to neuronal changes associated with schizophrenic patients. Other studies have shown decreases of BDNF or its corresponding receptor, Trk B, in the hippocampus of schizophrenics.

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Dwivedi et al. (2003) immunohistochemically stained hippocampi of postmortem human subjects and found that Trk B receptors were decreased in schizophrenia as compared to controls. Additionally, BDNF has been shown to be reduced in the prefrontal cortex in schizophrenics when compared to controls (Weickert et al., 2003). These studies are particularly important for cognitive function, as both the hippocampus and prefrontal cortex have been shown to play major roles in cognitive function (Fuster, 2002; Miller, Freedman, & Wallis, 2002; Sweatt, 2004).

Cognitive Deficits in Schizophrenia

In the schizophrenic population, cognitive disabilities are major and stable characteristics of the disease and are displayed in everyday functioning. These deficits have been linked to many areas of the brain involved in cognitive function, including the hippocampus, temporal cortex, frontal cortex, and striatum.

The Wisconsin Card Sorting Task is an important technique to evaluate cognitive deficits in human schizophrenic patients. Hartman, Steketee, Siva, Lanning, and McCann (2002) used this task in which participants were shown cards having individual characteristics of form, color, and number. They were presented with four cards and were then asked to sort the remaining 60 cards below one of the original four that matched their characteristics. The experimenter would respond "correct" when the subject responded for a characteristic. This procedure would continue until the subject had matched 10 consecutive cards correctly. However, without warning, the experimenter would change the characteristic that he wanted the cards to be sorted by and would continue until the subject had sorted the 10 consecutive cards according to the new characteristic. This continued until all cards had been sorted. Schizophrenic patients completed fewer categories and made more errors than control participants. These results further confirmed

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the original hypothesis that working memory would be impaired in schizophrenic patients.

Spatial working memory deficits in chronic schizophrenic patients have been examined by Okada (2002).   Twenty-two patients and 11 control participants were given two tasks. In the first task, participants were asked to trace the outline of an open square on a computer screen. After five squares were traced, a 25-second rest period occurred. A video recording device was turned on to record eye movements and the participants were asked to reproduce the same square four times with their eyes without the original outline on the screen. Results on this task showed that chronic schizophrenic patients had significantly greater difficulties in reproducing the outlines of the square than either schizophrenic patients or control participants. These results support the conclusion that working memory would be impaired in chronic schizophrenic patients compared to healthy, normal controls.

Braver, Barch, and Cohen (1999) conducted a meta-analysis examining cognitive function in schizophrenia and found that patients display a deficit in episodic memory function, which refers to the ability to encode or retrieve newly learned events.   Also, executive functioning in schizophrenics is lower than that of controls. These functions may include switching, planning, and dual-task coordination. These deficits may be even more prominent when patients are required to perform two tasks simultaneously or to alternate between two difference tasks (Smith et al., 1998). An overall finding among all tasks requiring memory function is that schizophrenic patients clearly display deficits compared to normal-functioning humans.

The Evolution of Pharmacological Treatments for Schizophrenia

In the 1940s, researchers first developed antihistamine drugs for allergy relief. Because these drugs caused drowsiness in patients, a French surgeon, Henri Laborit used one group of

these antihistamines to calm patients before going into surgery. The most popular drug used by surgeons was chlorpromazine. The ability of this drug to induce a calming effect among these patients provoked psychiatrists Jean Delay and Pierre Deniker to test these drugs on psychotic patients. Results were astounding in relieving symptoms of diseases such as schizophrenia, thus these drugs were implemented into the mental health field. In 1955, more than 500,000 psychotic patients were permanently hospitalized in the United States. Because of this development of the alternative use of antihistamines, by the 1980s fewer than 220,000 schizophrenic patients were institutionalized (Comer, 2001 p. 453; Julien 1998, p. 253).

Typical vs. Atypical Antipsychotics

After the discovery of the effectiveness of these drugs in psychotic patients, two classifications, phenothiazines (such as chlorpromazine) and butyrophenones (such as haloperideol or droperidol), quickly became effective methods of treatment for schizophrenic patients. These typical antipsychotics possessed the capability to relieve positive symptoms of schizophrenia such as delusions and hallucinations. However, as with other drugs, these breakthroughs did produce significant extrapyramidal motor side effects, or unwanted movements such as shaking, twisting, or restlessness. These drugs also lacked the ability to alleviate the negative symptoms of the disease such as alogia or social withdrawl (Julien, 1998, p. 245).

By the late 1990s, additional pharmacological breakthroughs offered schizophrenic patients even more hope for leading normal functioning lives (Julien, 1998, p.237). These new drugs are categorized as atypical antipsychotics because they have the ability to block not only dopamine, but also serotonin receptors, suggesting an additional role of the serotinergic system in schizophrenia. One benefit to atypical antipsychotics is their ability to treat patients who

experience negative symptoms such as blunted affect and social withdrawal, a characteristic that most typical antipsychotics could not alleviate. An especially important aspect of atypical antipsychotics is the decrease in extrapyramidal motor effects. In addition to being involved in memory functions, dopamine also plays a role in motor function. Therefore, because of atypical antipsychotics ability to block a higher percentage or dopamine receptors, these side effects may be decreased in schizophrenic patients. Because of the advantages of atypical antipsychotics, they have the capacity to be an effective drug for the treatment of schizophrenia, with the ability to treat as many as 85% of schizophrenic patients compared with the 65% aided by earlier traditional antipsychotics (Comer, 2001, p. 461).

A Promising Antipsychotic Agent: Olanzapine (Zyprexa)

One of the most promising and more commonly prescribed atypical antipsychtoic drugs, olanzapine (trade name: Zyprexa), gained FDA approval in 1996. Olanzapine combines a high affinity for dopamine$_2$ and serotonin$_{2A}$ blockade, both of which have been shown to be overactive in schizophrenic patients. In fact, olanzapine has been shown to block 40% to 60% of dopamine$_2$ receptors and 84% of serotonin 5-HT$_2$ receptors. A six-week clinical trial conducted by McConnell and Kinon (2004) found that olanzapine was superior to a control group on the Positive and Negative Symptoms Scale, the Brief Psychiatric Rating Scale, and the Clinical Global Impression. As with other atypical antipsychtoics, olanzapine has rarely been shown to produce extrapyramidal motor side effects. An additional advantage of olanzapine is that it has been shown to be effective in the treatment of bipolar disorder (Tohen et al., 1999) and in pervasive developmental disorder (Potenza, Holmes, Kanes, & McDougle, 1999). The major side effects induced by olanzapine are weight gain, sedation, orthostatic hypotension, and dizziness. The weight gain is greater than that seen with another atypical antipsychotic, risperidone, but less

than that of clozapine (Allison et al., 1999).

Olanzapine Effects on Human Cognition. One important and stable characteristic of schizophrenia is a deficit in cognitive function. Recent studies have shown that olanzapine appears to improve cognitive function. In 2001, Cuesta, Peralta, and Zarzuela analyzed effects of olanzapine on cognitive functions of schizophrenic patients when treated with olanzapine or typical neuroleptics such as risperidone. Twenty-eight outpatients who were diagnosed with schizophrenia were tested on several measures that examined executive, attention, and memory functions. Results demonstrated that the olanzapine group produced a significantly greater improvement of negative symptoms and verbal memory when assessed on the Stroop Color-Word Test, the Wisconsin Card Sorting Test, the Rey-Complex Figure Test, and the Trail Making Test. Results indicate that when patients who suffer from chronic schizophrenia use atypical antipsychotic drugs, such as olanzapine, improvements in cognitive abilities can be shown on tests requiring these skills compared to conventional neuroleptic drugs.

In another study designed to analyze the effects of olanzapine on cognitive function in schizophrenics, Harvey, Green, McGurk, and Meltzer (2003) treated 377 schizophrenic patients with risperidone or olanzapine. After eight weeks of treatment with the respective drugs, they were tested on a spatial memory task and the Wisconsin Card Sorting Task. For the spatial memory task, subjects viewed a presentation of a black circle on a computer screen. They were then subjected to a delay in which they engaged in a distracter task, which consisted of counting backward outloud. After the delay, the patients were presented with eight circles, each representing the possible target locations, and were asked to indicate the position of the original circle. The Wisconsin Card Sorting task again consisted of subjects matching a series of cards to a set of four target cards. Subjects were required to sort the remaining cards according to a

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similar characteristic. The results of these tasks revealed significant results compared with control groups. Patients treated with olanzapine for the eight-week period demonstrated improvements on cognitive functions when presented with tasks involving memory.

The mechanism through which olanzapine may work to improve cognitve function may be in its ability to enhance acetylcholinergic function in the brain which is believed to be depressed in schizophrenia brain (Bymaster, Felder, Ahmed, & McKinzie, 2002; Mihaiolescu & Drucker-Colin, 2000; Tracy, Monaco, Giobannetti, Abraham, & Josiassen, 2001). Shirazi-Southall, Rodriguez, and Nomikos (2002) conducted analyses to determine the effects of olanzapine on acetylcholine expression in rats. Microdialysis was conducted with either saline or olanzapine and results indicated that animals treated with olanzapine demonstrated a 1500% increase in acetylcholine release in the hippocampus. Because schizophrenics demonstrate decreases in the ACh system, these results support the idea that olanzapine's improvement of cognitive function in the schizophrenic population may be mediated through the ACh system.

Olanzapine Effects on Animal Cognition. Using information on the benefits of antipsychotics for schizophrenic patients, researchers involved in rodent models of schizophrenia have examined the effects of many antipsychoticdrugs. In 2003, Wolff and Leander examined cognitive impairments in animals treated with olanzapine on a delayed match-to-sample radial maze task. Animals were initially trained to search for food in an eight-arm maze. Once animals achieved stable performance, olanzapine was administered and then animals were tested again on the maze. Animals that were given olanzapine showed improved performance during the retention phase of the task. Mechanic, Maynard, and Holloway (2003) used the amphetamine model of schizophrenia on a place conditioning task. After being treated chronically with amphetamine, animals displayed a significant preference for the drug-paired side of the chamber.

After amphetamine-produced preference had been established, animals were treated with olanzapine which produced a significant preference shift. Thus olanzapine was responsible for preventing the expression of the amphetamine-induced conditioning. These results indicate that olanzapine may improve cognitive performance in animals.

<p style="text-align:center;">*Rodent Models of Schizophrenia*</p>

Animal models have been essential for examining the underlying mechanisms of schizophrenia in order to gain insights to the brain and its functions without conducting human experiments. Over the years, researchers have developed many models in which to explore such a complex disease, including the amphetamine, neonatal hippocampal lesion, and dopamine D_2 supersensitization models.

Amphetamine Model of Schizophrenia

One method researchers use to study schizophrenia is the alteration of the dopamine system through high acute doses of amphetamine administration. Amphetamine is a drug that increases the release of dopamine, blocks reuptake of dopamine through blockade of the dopamine transporter, and inhibits the presynaptic dopamine autoreceptor (Feldman & Weidenfeld, 1998). Validation of the amphetamine model of schizophrenia has been shown in past studies through analyzing sensorimotor gating. Auditory sensorimotor gating is severely disrupted in patients with schizophrenia and can be measured by assessing prepulse inhibition (PPI) of acoustic startle responses (Braff, et al., 2001; Hamm, Weike, & Schupp, 2001). PPI is the ability to inhibit a startle response to a loud acoustic stimulus (ranging from 110-120 dB) when it is preceded by a lower level prepulse (70-80dB). Studies have shown that PPI is disrupted in animals and in humans by stimulation of the dopamine system brain areas rich in dopamine, such as the nucleus accumbens (Clum & Hammer, 2004). For example, Tenn,

Fletcher and Kapur (2003) studied the effects of chronic amphetamine administration on PPI when animals were treated with high doses of D-amphetamine sulphate in rats, and administered the drug for 3 weeks. Animals were then left drug-free for 22 days followed by testing on a prepulse inhibition task. Before each trial, animals were given a challenge of either saline or amphetamine. Animals that were chronically treated with amphetamine followed by an amphetamine challenge showed a significantly lower percentage of PPI compared to saline treated animals. These results are consistent with human literature, and demonstrate that deficits in PPI may be related to hyperfunction of the dopaminergic system.

Neonatal Hippocampal Lesion Model of Schizophrenia

Schizophrenia has been associated with changes in brain structures. In schizophrenic patients, the volume of tissue in the hippocampus and frontal cortex has been shown to be lower than that of control adults and lateral ventricles have been shown to be enlarged (Beerpoot, Lipska & Weinberger, 1996; Keageles, Humaran & Mann, 1998; Soares & Innis, 1999). Based on the fact that the hippocampus has been shown to be important in spatial learning as well as social performance, several studies have examined cognitive and social deficits produced in animals with altered hippocampus structures to compare these changes with memory and social deficits in human schizophrenic patients.

In order to mimic the damage to this structure in human schizophrenics, many researchers have used an animal model in which a portion of or the entire hippocampus is lesioned early in development at postnatal day 7 (Lipska & Weinberger, 2002). When schizophrenic patients and lesioned animals were assessed on similar cognitive and social abilities, both groups demonstrated: 1) faster acquisition of classical conditioning, 2) increased generalization, 3) impaired spatial abilities, 4) deficits in using contextual information, 5) poor performance on

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complex tasks, 6) increased stereotyped behavior, 7) increased superstitious behavior, and memory deficits (Schmajuk, 1987; Schmajuk, 2001; Schmajuk, Christiansen & Cox, 2000).

Several studies also support the view that schizophrenic patients and hippocampal lesioned animals suffer from deficits in latent inhibition. Latent inhibition is a classical conditioning learning phenomenon in which pre-exposure to the conditioned stimulus reduces its subsequent conditioning when the conditioned stimulus is paired with an unconditioned stimulus (Lubow & Moore, 1959). In numerous evaluations of both schizophrenic patients and hippocampally-lesioned animals, both groups demonstrated 1) high arousal levels, 2) poor habituation of the orienting response, and 3) event-related potentials elicited by unpredicted stimuli, thus confirming the role of the hippocampus in learning and how any change to the structure, whether in humans or animals, may result in decreased learning abilities (Schmajuk, 1987; Schmajuk, 2001).

Social withdrawal and isolation are two characteristics of negative symptoms in schizophrenia. Sams-Dodd, Lipska, and Weinberger (1997) examined the effects of neonatal ibotenic acid lesions of the ventral hippocampus on social behavior. Animals were lesioned at postnatal day seven (P7) by an infusion of ibotenic acid into the ventral hippocampal formation. Animals were then raised to adolescence and then tested in the social interaction test. In this procedure, animals with identical lesion treatment but had not been housed together were placed into an arena 80-90 cm apart and were measured for distance traveled and for active/passive social interaction. Animals given hippocampal lesions at P7 with ibotenic acid demonstrated significantly less time in the central zone as well as were less engaged in active social interaction when compared to shams, supporting the hypothesis that the hippocampus may be involved in these negative symptoms of schizophrenia. Repeatedly, the role of the hippocampus has been

linked to cognitive deficits in schizophrenic patients. Using this rodent model, researchers have been able to model these effects in order to further study the role of cognition in schizophrenia as well as create a treatment for the disease.

The amphetamine reward and hippocampal lesion animal models have been widely used to examine deficits that are presented in schizophrenic patients. However, both of these models have significant weaknesses. Although the amphetamine model does produce a significant increase in dopamine production, this change is only acute as it lasts only as long as the psychoactive effects of the high dose of amphetamine. Because this method does not permanently alter the dopaminergic system, this is an obvious weakness as a model for schizophrenia.

The hippocampal lesion model is highly accepted as a valid model in the research of schizophrenia, as human schizophrenic patients demonstrate alterations in the hippocampus. However, a weakness in this model is that a complete and consistent lesion of the hippocampus is difficult to achieve at postnatal day 7 due to the consistency of the tissue and the diminutive structure. At a recent conference, a researcher pointed out the difficulty of obtaining consistent lesions in this model of schizophrenia (Bryan Kolb, personal communication, November 18, 2003). Thus, although this model produces some similar behavioral deficits as observed in schizophrenia, its primary weakness may lie in the inconsistent ablation and behavioral results, as well as failing to simulate dopamine hypersensitivity known to be prevalent in schizophrenia.

Dopamine D_2 Supersensitization Model of Schizophrenia

Because of the shortcomings in these previous models, the dopamine D_2 receptor suspersensitization model used in this laboratory may be a more effective model of schizophrenia. In this model, the dopamine D_2 receptor is supersensitized through neonatal

quinpirole administration during the first three weeks of life in a rat. A critical period of brain development occurs during the first few weeks of life. If any damage or change is made through administration of a drug or a manipulation of the environment, the effects often result in permanent brain damage (Kolb & Whishaw, 2001). Therefore, this model takes advantage of producing a change in the dopamine system during this critical period of brain development to cause a permanent change in behavior, as is observed in schizophrenia.

Dopamine binds to two families of receptors, the D_1 and the D_2. Each of these families has receptor subtypes. For example, the D_1 family has the D_1 and D_5 subtype, and the D_2 family has the D_2, D_3, and D_4 subtypes. Quinpirole acts as an agonist to the dopamine D_2/D_3 receptor, which means that it substitutes for dopamine at the D_2 and D_3 receptors. Animals neonatally treated with quinpirole display hyperlocomotion, increased yawning, paw treading, and vertical jumping, all which are related to increases in dopamine D_2 receptor sensitivity (Kostrzewa & Brus, 1991; Kostrzewa, Hamdi, & Kostrzewa, 1990). However, dopamine D_2 receptor hypersensitivity is verified through an acute injection of quinpirole given when the rat is an adult (approximately P60). Animals primed with quinpirole typically demonstrate a three to four-fold increase in yawning and vacuous chewing; both behaviors mediated by the dopamine D_2 receptor (Cooper, Rusk, & Barber, 1989). Additionally, when these animals are treated with haloperidol or nicotine after neonatal quinpirole treatment, all of these behavioral changes are reversed (de Bruin, Ellenbroek, van Luijtelaar, Cools, & Stevens, 2001; Tizabi, Copeland, Brus, & Kostrzewa, 1999). The advantage of this model is its ability to produce permanent changes neurochemically in the dopamine system.

Cognitive Impairments Produced by Neonatal Quinpirole Treatment. Increase in the sensitivity of dopamine receptors has been shown to produce cognitive deficits (Braver et al.,

1999). In this laboratory, we have demonstrated that neonatal quinpirole treatment produces cognitive deficits in rats behaviorally tested on the Morris Water Task. Brown et al. (2002) treated animals from postnatal days 1 to 11 with quinpirole (50 μg/kg) or saline. At postnatal day 60 (P60), the adult animals began training on the Morris Water Task (MWT). The MWT is a spatial memory task in which an animal uses extramaze cues in order to locate a hidden platform. The platform is located approximately 1 cm below the water surface, and the water is colored opaque with powdered milk or powdered black paint. Animals were trained on the place task, in which the platform remains stationary, for 24 trials over three days. Immediately after the last training trial, a probe trial was administered in which the platform was removed. Animals were assessed on two dependent measures: acquisition latency, which is the amount of time it takes for the animal to locate the platform and the mean search difference (MSD) score of the probe trial, which is the ratio of time spent in the quadrant previously containing the platform versus the other three quadrants. Results showed that rats neonatally treated with quinpirole displayed a deficit on the probe trial compared to rats neonatally treated with saline, indicating that neonatal treatments of a dopamine agonist produces cognitive deficits on tasks requiring spatial memory.

In a different study (Brown et al., 2004), animals were administered nicotine in adulthood to attempt to alleviate the cognitive deficits produced by neonatal quinpirole treatment. In this study, animals were neonatally treated with quinpirole (1mg/kg) or saline from postnatal day 1 to 21. On postnatal day 60, D_2 supersentization was verified through yawning and animals treated with quinpirole demonstrated a five-fold increase in yawning compared to controls. Animals were then treated twice daily with nicotine or saline for 14 days. The rationale for using nicotine in adult animals was that approximately 75% of schizophrenic patients smoke cigarettes, which is about 50% higher than the normal population (Le Duc & Mittleman, 1995). Nicotine is a

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psychoactive drug that acts as an acetylcholine agonist and has the ability to enhance cognition in schizophrenic patients (Adler et al., 1998). One day after the final nicotine treatment, animals began testing first on the place and second on the matching-to-place versions of the Morris Water Task. In the matching-to-place task, animals are given two trials per day in which the platform is placed in a new random quadrant each day. Animals are released from the most distant point from the platform each day. Animals treated with quinpirole demonstrated significant cognitive deficits on both versions of the MWT, which were eliminated by nicotine treatment. This further demonstrates the ability of neonatal quinpirole treatment to cause cognitive dysfunction; however, nicotine reversed these affects.

Brain tissue was removed and the hippocampus, frontal cortex, and striatum were dissected away from the rest of the brain. Nerve growth factor (NGF) and brain-derived growth factor (BDNF) were analyzed through an ELISA analysis, and choline acetyltransferase (ChAT) was analyzed using a radioimmunoassay. Results showed that animals neonatally treated with quinpirole demonstrated significant decreases in NGF and ChAT in the hippocampus as well as marginally significant decreases in hippocampal BDNF, all of which were partially alleviated by nicotine. This seems to indicate that not only does D_2 supersensitization produce cognitive deficits, but nicotine, an ACh agonist, eliminates cognitive deficits and partially alleviates decreases in NGF and BDNF, two neurotrophic factors known to be highly related to ACh activity in the septohippocampal pathway. Therefore, this supports the hypothesis that decreases in the hippocampal ACh system are at least partially responsible for deficits in spatial memory produced by neonatal quinpirole treatment.

Brown et al. (2004) demonstrated that neonatal quinpirole treatment from P1-21 produces early developmental deficits in spatial memory in animals in animals tested on the Morris Water

Task from P22-28. These deficits were once again eliminated by acute adult treatment with eticlopride, a dopamine D_2 antagonist. These animals also demonstrated an increase in plasma corticosterone levels, which are known to produce decreases in neurotrophic factors in adult rats. This study seems to indicate that the D_2 receptor seems to be mediating cognitive deficits produced by neonatal quinpirole treatment.

Gender Differences and D_2 Supersensitization. In female rats, estrogen acts to inhibit GABA neurons in the striatum and accumbens, which in turn increases dopamine function. Also, estrogen displays the ability to enhance dopamine release by down-regulating dopamine D_2 receptor function (Becker, 1999). When the dopamine system is sensitized in this manner, the effects of the receptor may be magnified.

The estrous cycle of the female rat has three stages, and appears to recur every four days (Nelson, 1995). The stage of the estrous cycle can be determined through swabbing cells from the vaginal lumen after sterile wash then examining these cells microscopically. Vaginal estrous lasts 36 hours, and cornified epithelial cells are present. Vaginal estrous is followed by a period during which cornified cells become reduced in number, and this stage is called vaginal diestrous, and has a duration of 48 hours. The first day of diestrous is referred to as diestrous I, and the second day is referred to as diestrous II. The next phase is characterized by the presence of many nucleated epithelial cells and this stage is vaginal proestrous, which lasts for approximately 12 hours (Nelson, 1995). It is important to understand that behavioral estrous coincides with vaginal proestrous, and there is elevated estrogen secretion during this period as well as estrous behavior. Coincident with the endogenous surges of estrogen and progesterone during behavioral estrous, there is enhanced dopaminergic activity, as indicated by enhanced dopamine release, metabolism, and reuptake (Becker, 1999).

In conclusion, the literature confirms that not only do cognitive disabilities exist in schizophrenic patients, but also that antipsychotics may improve or reverse these effects. Additionally, in an attempt to create a rodent model of this disease, research has found that treatments with dopamine agonists such as amphetamine or quinpirole can induce this kind of dysfunction in animals. Similar to the human literature, when these animals are treated with atypical antipsychotics, they demonstrate improvement on cognitive tasks.

Statement of the Problem

The current study was designed to produce cognitive disabilities in animals through neonatal treatment with quinpirole and then attempt to reverse these effects in animals through treatment with olanzapine in adulthood. From postnatal days 1 to 21, animals were treated with quinpirole (1mg/kg) once a day. Yawning behavior was tested at P60 to verify dopamine D_2 supersensitization. At postnatal day 61, animals began olanzapine treatment twice daily for four weeks to simulate the dosing schedule in human schizophrenics. Following the last day of olanzapine administration (P 90), animals began behavioral testing on the place and match-to-place versions of the MWT for the next seven consecutive days. Finally, on P98, yawning behavior was retested through acute administration of quinpirole (1mg/kg). On P99, tissue was harvested and NGF & BDNF analyses were conducted.

It is important to note that no drugs were administered when behavioral testing took place. Therefore, the neurophysiological effects of drug treatment were solely responsible for any behavioral changes observed on the MWT. Acquisition latencies were scored as dependent measures on both the place and match-to-place versions of the task. This is the amount of time required for the animal to reach the platform. Mean search difference (MSD) and mean zone difference (MZD) scores of the probe trial were scored on the place version. The MSD score is

the ratio of the time the animal spent searching in the quadrant formerly containing the platform (Target, T) in comparison to the other three quadrants that never contained the platform (W, X, and Y). The MZD score is the ratio of the number of visits to the former platform (Target, T) location to three other zones in the pool of equivalent size located in each of the three other quadrants (W, X, and Y). This provides a ratio of the amount of time or number of visits to the former platform location versus other zones in the pool. The higher the score on the MSD and MZD measures, the stronger the bias to the former platform location. These measures have been utilized in several other studies from this laboratory (Brown, Gonzalez, & Kolb 2000; Brown, Gonzalez, Whishaw, & Kolb, 2001; Brown, et al., 2002; Brown et al., 2004a).

Assignment to Groups

There were four treatment groups: the first drug representing neonatal drug treatment and the second drug representing adult drug treatment: quinpirole/saline, quinpirole/olanzapine, saline/olanzpine, and saline/saline. Males and females were represented in each group.

CHAPTER 2

METHODS

Experiment 1

Subjects

Eight female and 4 male Sprague-Dawley rats were obtained from Harlan Laboratories, Inc. (Indianapolis, ID) and mated in plastic polycarbonate cages. The offspring of these breeders were used in this experiment. From these litters, 43 female and 35 male Sprague-Dawley rats were used in this study. Day of birth was counted as P0. Rats were weaned at P21, and after weaning, all animals were housed in groups according to the same gender and same neonatal drug treatment group. Animals were housed in a climate controlled environment with 12 hours of light and 12 hours of dark with food and water available ad libitum.

Apparatus

The MWT consisted of a galvanized tub 145 cm in diameter and 58.4 cm in height. The inside of the pool was painted black and filled with water to a height of approximately 40 cm with a temperature of 19° to 21° C. A platform composed of white plastic PVC pipe with a diameter of 12.7 cm was placed in the southwest quadrant of the pool approximately one cm below the surface of the water. The water in the tub was rendered opaque by dissolving black nontoxic tempera paint in the water to obscure the platform location. The pool was surrounded by several extramaze cues including posters hung on the surrounding walls, a video monitor placed on a cart at the east side of the tub, two waste baskets placed on the west side of the tub, and the experimenter sitting at the south point of the tub who was always dressed in a beige lab coat. The overhead lights were turned off and four spotlights, attached to poles at tub level or lower, illuminated the room.

Procedure

Drug Administration. Animals were injected i.p with Quinpirole HCl (1 mg/kg) or saline (0.9%, 1 mg/kg) from postnatal days one to 21 (P1-21). Rats were raised to adulthood (P60) and one day after yawning behavior was observed and recorded, rats were injected i.p. with Olanzapine (3 mg/kg) or saline (0.9%, 1 mg/kg) from postnatal days 62-90.

Yawning Behavior. Yawning behavior was observed at P61 before and again at P98 after chronic olanzapine treatment and behavioral testing was complete. For yawning, all animals, regardless of neonatal treatment, were injected i.p. with quinpirole (1mg/kg) and placed in an empty cage. Number of yawns and vacuous chewing movements were counted for one hour.

Morris Water Task-Place Version. Twenty-four hours after their last olanzapine injection, P91, animals were tested on the MWT. Drug administration groups and sex groups were divided into four randomly selected groups. The animals were given two trial blocks of four trials each per day. The animals were tested at approximately the same time for three consecutive days, yielding a total of 24 trials.

The animals were released once from each of the four release points (north, south, east, or west) on each trial block. They were allowed 60 seconds to reach the platform. If the animal did not reach the platform, the experimenter placed it on the platform. Regardless of if the animal reached the platform or was placed on the platform by the experimentor, it spent the last 10 seconds of each trial on the platform. Acquisition latency was recorded in each training trial which was defined as the amount of time from when the rat is released into the pool until the time it reached the platform.

Immediately after the last training trial on the final day of training, animals were given a probe trial in which the platform was removed from the pool. Animals were released from the

North release point and allowed to swim for 60 seconds. Swim patterns were recorded by a CCD video camera (Rockhouse Products, NJ) which was mounted above the pool. Mean Search Difference and Mean Zone Difference scores were recorded from an analyses of swim patterns on the video recorded during the probe trial.

Morris Water Task-Match-to-Place Version. The day following the last day of the place version of the Morris Water Task (P94), animals were trained on the match-to-place version. Animals were given two trials per day for four days for a total of eight trials. Each day the platform was placed in one of the four quadrants (NE, SE, SW, or NW) and animals were released from the most distant point from the platform. Following the procedure for the place version, the animals were allowed 60 seconds to reach the platform. If the animal did not reach the platform, the experimenter placed it on the platform, where it remained for 10 seconds. Immediately after their first attempt to reach the platform, animals were re-released from the same point and again allowed 60 seconds to locate the platform. When the animal located the platform or if the animal did not reach it, the animal was removed from the pool. This procedure was repeated for three additional days. Just as with the place version, acquisition latency was recorded.

Experiment 2

In the first experiment, it was found that chronic olanzapine treatment eliminated cognitive deficits in animals neonatally treated with quinpirole on both probe trial measures as well as the match-to-place version of the MWT. Possibly more important was that chronic olanzapine completely reversed dopamine D_2 supersensitization as measured through yawning. Dopamine D_2 supersensitization is likely responsible for the observed performance deficits on the MWT. However, there is no information as to whether there are correlating changes in NGF

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or BDNF. Experiment 2 was designed to analyze NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor) which are both important neurotrophic factors in the development and maintenance of the septohippocampal pathway.

Subjects

Brain tissue was taken at P98 from animals used in Experiment 1, frozen immediately in cold isopentane (-20 C), and stored in a -80 C freezer. Three brain areas were later dissected away from the rest of the brain: the hippocampus, the frontal cortex, and the cerebellar cortex, and were analyzed for NGF and BDNF.

Procedure

Neurotrophic Factors Analysis. The hippocampus, frontal cortex, and cerebellum were analyzed. The BDNF or NGF E_{MAX} immunoassay system protocol were followed (Promega, Madison, WI). In brief, 10 μl of the Anti-BDNF mAb was added to 9.99 ml of carbonate coating buffer (pH 9.7). One hundred μl of this mixture was added to each well of a polystyrene ELISA plate and incubated overnight at 4° C. All wells were washed with a TBST wash buffer and incubated at room temperature for one hour. Nonspecific binding was blocked through adding a block and sample 1X buffer and deionized water mixture to each well and incubated at room temperature for 1h. The BDNF standard curve was prepared using the BDNF standard supplied from the manufacturer (1μg/ml). The standard was diluted 1:2,000 in Block x Sample 1 x Buffer to achieve a concentration of 500pg/ml. Six 1:2 serial dilutions were prepared and incubated with shaking at room temperature for 2h. Anti-Human BDNF pAB was then added to each well plate, incubated at room temperature (2h), which was followed by incubation (1h) with Anti-IgY HRP conjugate. Finally, to each well 100 μl of TMB one solution was added and incubated with shaking for 10 min at room temperature. The entire reaction was stopped in each well with the

addition of 100µl of 1N hydrocholoric acid, and read 30 min later.

A very similar protocol for NGF analysis was followed, and the Promega kit for NGF (NGF E_{MAX} system; Promega, Madison, WI) was used for this purpose. For NGF analysis, well plates were coated with Anti-NGF Polyclonal Antibody (pAb) which binds soluble NGF. The captured NGF was bound by a second specific monoclonal antibody (mAb). After washing, the amount of specifically bound mAb was then detected using a species-specific antibody conjugated to horseradish peroxidase (HRP) as a tertiary reactant. The unbound conjugate was removed by washing and following incubation with a chromogenic substrate, the color change was measured. Both neurotrophic factors were quantified by measuring the amount of neuotrophic factor in pg/mg of tissue, which was measured by absorbency of the signal by the plate reader (BioRad instruments).

Research Design

The design variables were neonatal drug treatment (quinpirole, saline), adulthood drug treatment (olanzapine, saline), and sex (male, female). For the yawning behavior measure, a 2 x 2 x 2 four-way design with repeated measures on number of yawns, P61 and P98 was used. For the acquisition latency-place version measure was scored as a 2 x 2 x 2 x 6 mixed design with repeated measures on 6 trial blocks. For the Mean Search Differences-place version measure, a 2 x 2 x 2 randomized design was used. For the Mean Zone Differences-place version measure, a 2 x 2 x 2 randomized design also used. Acquisition latency-match-to-place version measure was a 2 x 2 x 2 x 4 four-way ANOVA with repeated measures on pairs of trials, expressed as Trial 2 minus Trial 1.

Hypotheses

Yawning Behavior

Hypothesis 1. Neonatal quinpirole will produce a decline in yawning behavior versus animals neonatally treated with saline, replicating previous work by Kostrzewa et al. (1995) and Brown et al., 2004b).

Hypothesis 2. However, an increase in yawning will be reversed in animals given adulthood olanzapine treatment.

Morris Water Task-Standard Version

Hypothesis 3. Animals neonatally treated with quinpirole will demonstrate a deficit on the MWT relative to saline control animals, replicating previous work (Brown et al., 2002; 2004a).

Hypothesis 4. These deficits will be reversed by olanzapine treatment in adulthood and animals will perform equal to saline controls.

Morris Water Task-Match-to-Place Version

Hypothesis 5. Animals neonatally treated with quinpirole will demonstrate higher acquisition latencies on trials 1 and 2, and saline control animals will demonstrate a decrease in latency from trial 1 to trial 2, which will demonstrate a deficit in rats neonatally treated with quinpirole. These results will be reversed by olanzapine treatment in adulthood and animals will perform equal to saline controls.

Neurochemical Analyses

Hypothesis 6: Neonatal quinpirole treatment will produce a significant decrease in nerve growth factor (NGF) replicating previous work (Brown et al., 2004a; 2004b). This decrease will be alleviated by chronic olanzapine. No significant differences in NGF in the frontal cortex are

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predicted.

   Hypothesis 7: Neonatal quinpirole treatment will produce a marginally significant decrease in brain-derived neurotrophic factor (BDNF) in the hippocampus, replicating previous work (Brown et al., 2004a; 2004b). This decrease will be alleviated by chronic olanzapine. No significant differences in the frontal cortex BDNF are predicted.

CHAPTER 3

RESULTS

For all results, the values of the variables will be represented as follows: F=Female,

M=Male, Q=Quinpirole, O=Olanzapine, and S=Saline.

Experiment 1

Yawning Behavior, Postnatal Day 61

A four-way analysis of variance (ANOVA) revealed a significant main effect of sex,

$F(1,68)=34.02$, $p < .0001$, and developmental drug treatment, F $(1,68)=6.359$, $p <.01$ (see Figure

1). Post hoc analyses revealed that Male-Quinpirole yawned more often than Male-Saline and

Female-Quinpirole yawned more often than Female-Saline. This result demonstrates that

neonatal quinpirole treatment produces D_2 supersensitization compared to rats neonatally treated

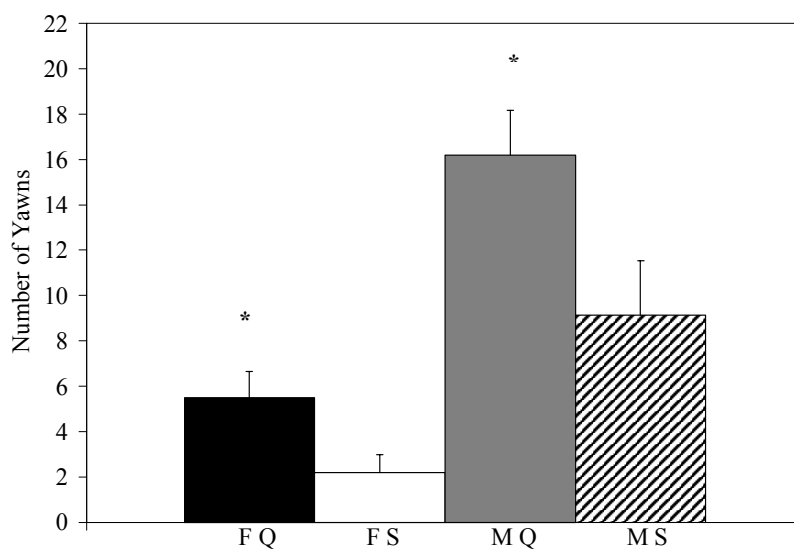with saline. Sample sizes: FQ=19, FS=21, MQ=17, MS=14.

Figure 1. Yawning Behavior, Postnatal Day 61

Yawning Behavior, Postnatal Day 98

A four-way analysis of variance (ANOVA) was conducted and revealed significant main effects of sex, $F_{(1,68)}=5.95$, $p<.01$, and adult drug treatment, $F_{(1,68)}=8.07$, $p<.006$ (see Figure 2). Post hoc analysis revealed that Female-Quinpirole-Saline displayed a 4-fold increase in yawns compared to Female-Saline-Saline, Female-Quinpirole-Olanzapine, and Female-Saline-Olanzapine. Sample sizes: FQO=9, FQS=10, FSO=10, FSS=11, MQO=8, MQS=9, MSO=9, MSS=5.
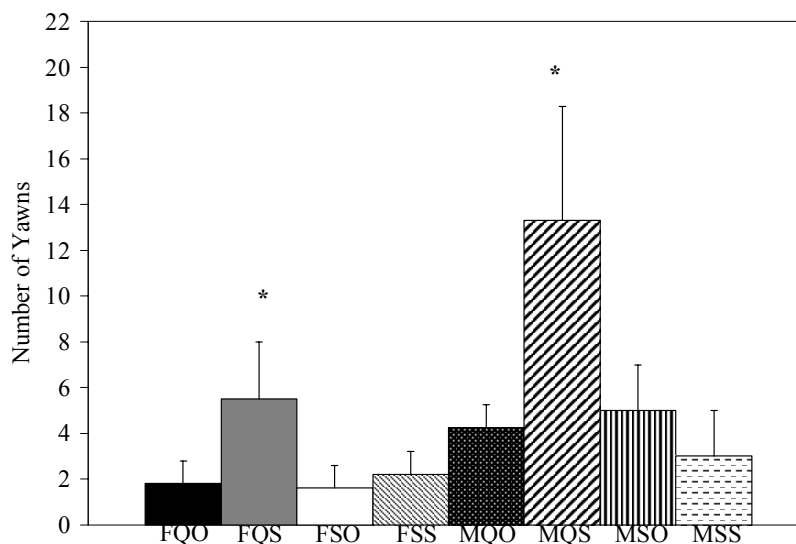
Figure 2. Yawning Behavior, Postnatal Day 98

Morris Water Task: Place Version

Acquisition Latency. A four-way analysis of variance (ANOVA) was conducted for male and female animals separately (see Figures 3a and 3b). Male results revealed a significant main effect of trial block, $F_{(1,30)}=51.73$, $p<.0001$. Female results revealed a significant interaction of

trial block X adult drug treatment, F (1, 36)=3.713, p<.003. Sample sizes: FQO=9, FQS=10, FSO=10, FSS=11, MQO=8, MQS=9, MSO=9, MSS=5.
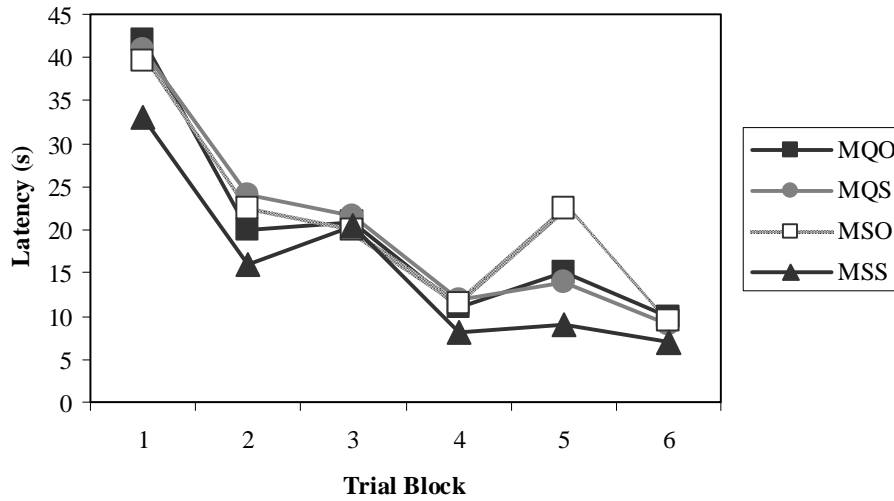
Figure 3a. Morris Water Task: Place Version, Acquisition Latency, Males
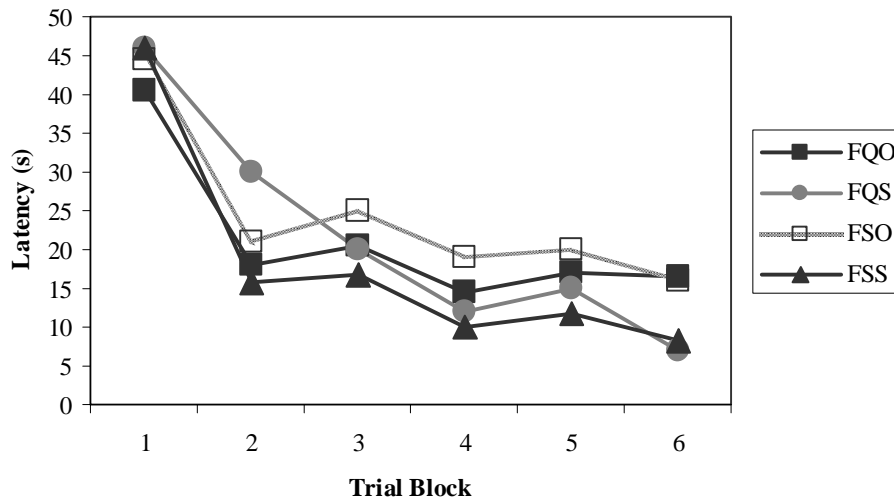
Figure 3b. Morris Water Task: Place Version, Acquisition Latency, Females

Mean Search Differences. A three-way analysis of variance (ANOVA) was conducted and revealed a significant interaction of developmental drug treatment X adult drug treatment, F (1, 66)=6.64,p<.01 (see Figure 4). Sample sizes: QO=17, QS=19, SO=19, SS=16.
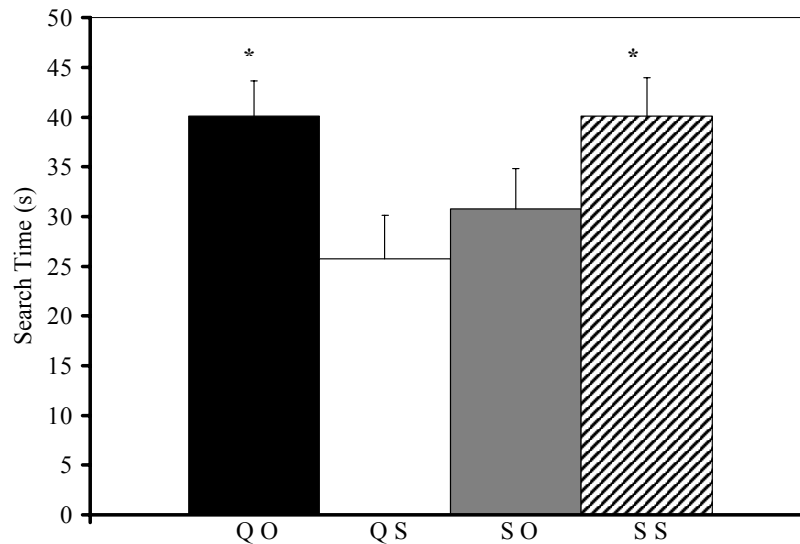
Figure 4: Morris Water Task: Place Version, Mean Search Differences

Mean Zone Differences. A three-way analysis of variance (ANOVA) was conducted and revealed a significant interaction of developmental drug treatment X adult drug treatment, F (1,66)=8.84,p<004 (see Figure 5). Sample sizes: QO=17, QS=19, SO=19, SS=16.
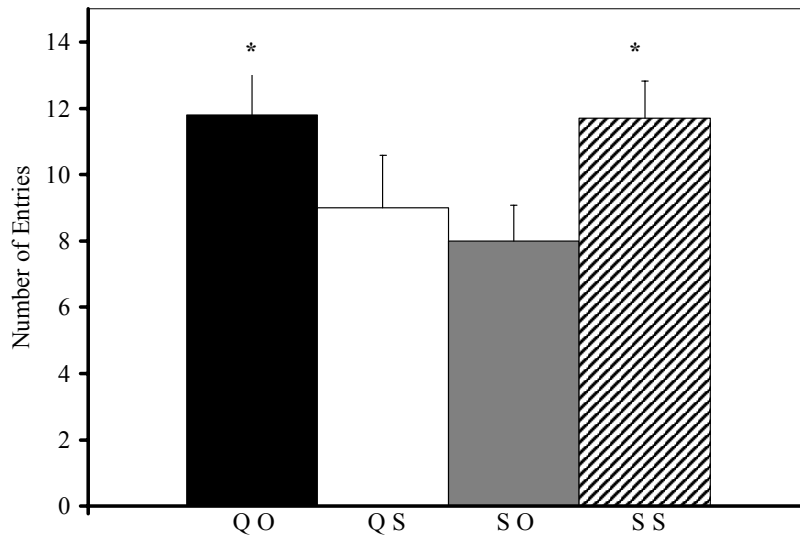
Figure 5: Morris Water Task: Place Version, Mean Zone Differences

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Morris Water Task: Match-to-Place Version

    Acquisition Latency. A four-way analysis of variance (ANOVA) was conducted and revealed a main effect of sex, $F(1,64)=4.48, p<.03$, and a significant interaction of developmental drug treatment X adult drug treatment, $F(1,64)=4.48, p<.03$ (see Figure 6). Post hoc analyses revealed that Female-Quinpirole-Olanzapine animals had higher acquisition latencies than Female-Quinpirole-Saline, Female-Saline-Olanzapine, and Female-Saline-Saline. Post hoc analyses also revealed that Male-Quinpirole-Olanzapine and Male-Saline-Saline had higher acquisition latencies than Male-Quinpirole-Saline or Male-Saline-Olanzapine. Sample sizes: FQO=9, FQS=10, FSO=10, FSS=11, MQO=8, MQS=9, MSO=9, MSS=5.
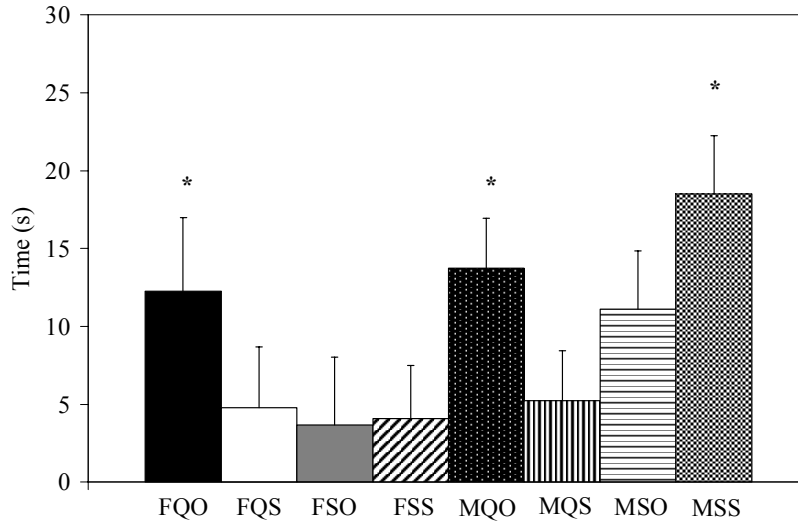
Figure 6: Morris Water Task: Match-to-Place Version, Acquisition Latency

Experiment 2

Nerve Growth Factor Analysis

NGF in the Hippocampus. A four-way analysis of variance (ANOVA) was conducted

and revealed significant main effects of neonatal drug treatment, $F(1,32)=.66, p<.42$ and

adulthood drug treatment, $F(1,32)=3.46, p<.07$ (see Figure 7). The analysis also showed an

interaction of neonatal drug treatment X adulthood drug treatment, $F(1,32)=4.54, p<.04$. Post hoc

analyses revealed that the Quinpirole-Saline, Saline-Olanzapine, and Saline-Saline had higher

levels of NGF in the hippocampus. Sample sizes: QO=11, QS=7, SO=9, SS=9.
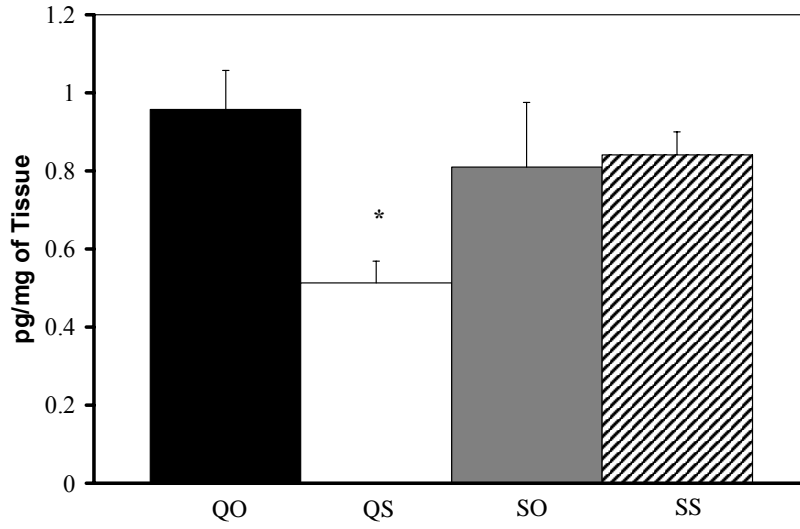
Figure 7. NGF in the Hippocampus

NGF in the Frontal Cortex. A four-way analysis of variance (ANOVA) was conducted

and revealed no significant effects (see Figure 8). Sample sizes: QO=11, QS=7, SO=10, SS=8.

Figure 8. NGF in the Frontal Cortex

NGF in the Cerebellum. A four-way analysis of variance (ANOVA) was conducted and

revealed on significant results (see Figure 9). Sample sizes: QO=11 QS=7, SO=10, SS=7.

Figure 9. NGF in the Cerebellum

Brain-Derived Neurotrophic Analysis

     BDNF in the Hippocampus. A four-way analysis of variance (ANOVA) was conducted and revealed no significant effects (see Figure 10). Sample sizes: QO=11, QS=7, SO=9, SS=9.

Figure 10. BDNF in the Hippocampus

     BDNF in the Frontal Cortex. A four-way analysis of variance (ANOVA) was conducted and revealed a significant main effect of neonatal drug treatment, $F_{(1,32)}$-5.39,p<.02 (see Figure 11). Sample sizes: QO=11, QS=7, SO=10, SS=8.
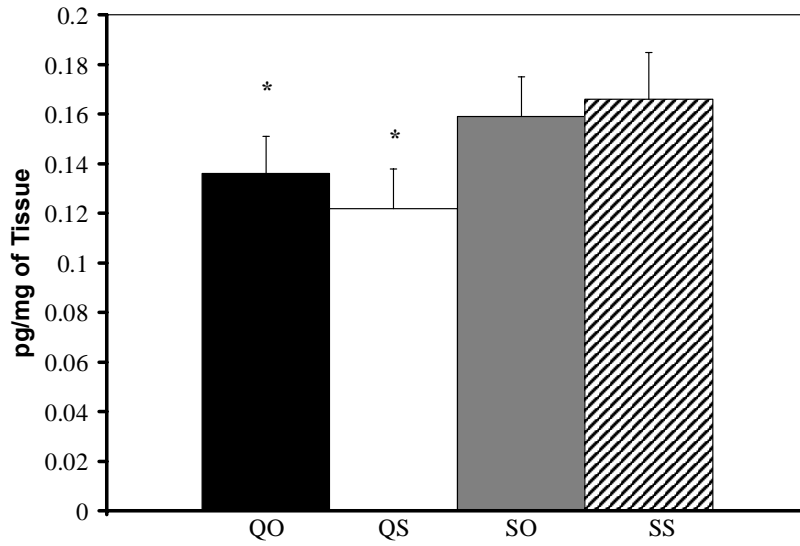
Figure 11. BDNF in the Frontal Cortex

BDNF in the Cerebellum. A four-way analysis of variance (ANOVA) was conducted and revealed no significant effects (see Figure 12). Sample sizes: QO=11, QS=7, SO=10, SS=7.
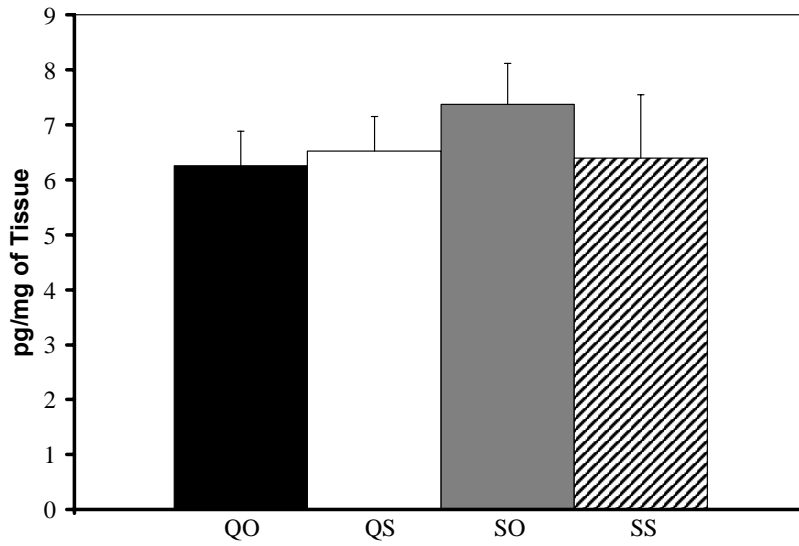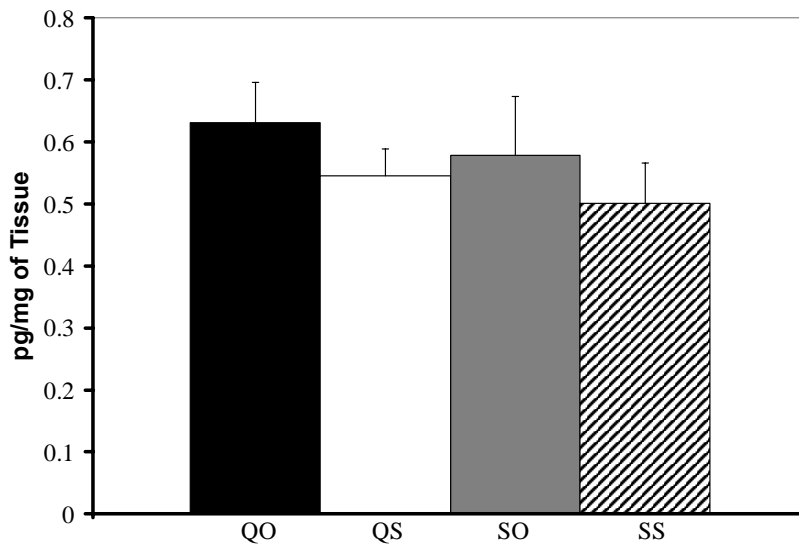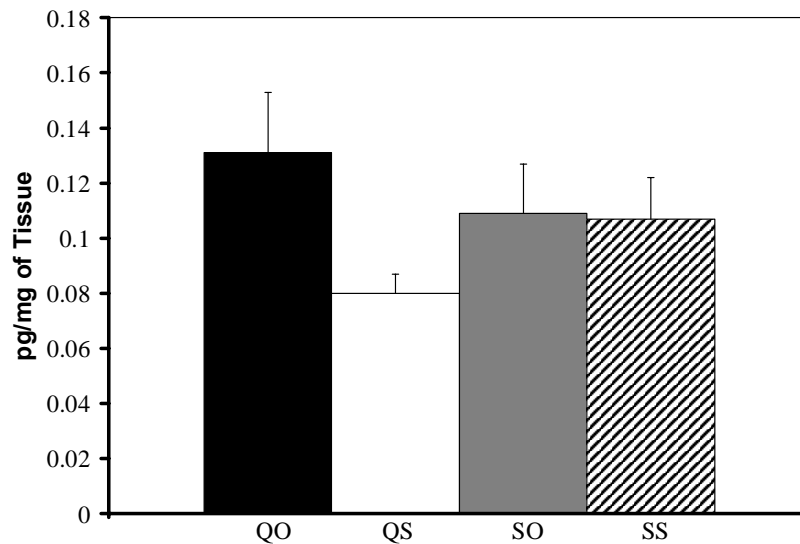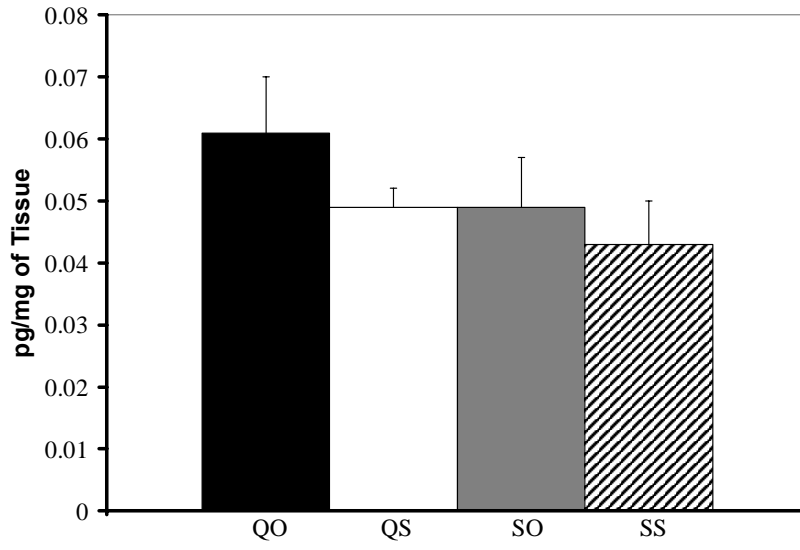
Figure 12. BDNF in the Cerebellum

CHAPTER 4

DISCUSSION

The results of this study demonstrated that (1) animals neonatally treated with quinpirole demonstrated a significant increase in yawning in adulthood that was alleviated by olanzapine treatment; (2) animals neonatally treated with quinpirole displayed cognitive deficits in the Morris Water Task, replicating previous work from this laboratory (Brown et al. 2002; 2004a; 2004b); (3) Adulthood olanzapine treatment in adulthood eliminated deficits produced by neonatal quinpirole treatment on the both the Mean Search Difference and Mean Zone Difference probe trial dependent measures of the MWT place version; (4) Adulthood olanzapine treatment in adulthood eliminated cognitive deficits on the match-to-place version of the MWT, but only in males; (5) Females did not demonstrate a significant deficit on the match-to-place version of the MWT; (6) Adulthood olanzapine treatment alleviated the significant decrease in NGF in the hippocampus produced by neonatal quinpirole treatment; (7) Neonatal quinpirole treatment produced a significant decrease of BDNF in the frontal cortex that was not alleviated by olanzapine. Essentially, the cognitive deficits produced by neonatal quinpirole treatment were eliminated by chronic olanzapine treatment. Importantly, this alleviation of cognitive deficits occurred after olanzapine treatment had ceased, and it appears that the alleviation of cognitive deficits may be mediated through olanzapine-induced alleviation of decreases in hippocampal NGF. Therefore, it appears that olanzapine may be producing a durable change in D_2 receptor or acetylcholinergic.

Possibly the most important finding in the current study is the ability of olanzapine to alleviate the increase in yawning of animals neonatally treated with quinpirole. This change demonstrates not only that the cognitive deficits seem to be mediated by D_2 supersensitization,

but that olanzapine has the ability to change the sensitivity of this receptor. This is based on the fact that olanzapine alleviated this increase of yawning after an 8-day washout and appears to be at least a semi-permanent effect. Therefore, based on the fact that olanzapine was able to alleviate D_2 supersensitization and cognitive impairment suggests that the change in sensitivity of the D_2 receptor may be responsible for the cognitive deficits observed in the current study.

Olanzapine alleviated the significant decrease of NGF in the hippocampus induced by neonatal quinpirole treatment. The neurotrophic factors NGF & BDNF play an essential role in neuronal maintenance and growth, as well as influence cognition. Both of these neurotrophins have been shown to enhance the release of Ach in the hippocampus (Knipper et al., 1994; Rylett, Goddard, Schmidt, & Williams, 1993; Schimode, Uek,i & Morita, 2003). In a study conducted by Sinson, Voddi, and McIntosh (1995) animals were given lateral fluid-percussion brain injuries and were then treated with NGF. Animals were then evaluated on the Morris Water Task and animals treated with NGF infusions performed significantly better than non-treated animals. Similarly, Cirulli, Berry, Chiarotti and Alleva (2004) trained animals for one day one the Morris Water Task. On the second day, animals were treated with BDNF injections into the hippocampus. The animals treated with BDNF showed a shorter latency and path length to reach the platform. These findings are consistent with the ability of neurotrophins to improve cognition. NGF and BDNF are also essential in neuroprotection in that they are related to the formation of new synapses. Schizophrenic patients do not demonstrate a change in the total number of hippocampal neurons but a decrease in the density of the neurons (Harrison, 2004). Therefore, NGF & BDNF play a vital role in the maintenance and growth of neurons.

Both NGF and BDNF work through similar mechanisms and can influence neuronal function temporarily or produce more permanent changes. NGF is known to bind to the tyrosine

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kinase A (trk A) receptor, and BDNF binds to the trk B receptor. Several studies have shown that ChAT positive neurons in the hippocampus have both trk A and trk B receptors in the cytoplasm. When these neurotrophic factors bind to their respective receptors, a series of events occur to produce changes in neuronal function. When the trk receptors bind to these neurotrophins, they are phosphorylated, which can produce changes in signal transduction pathways to phosphorylate proteins within the cell, including transduction pathways that may produce changes in protein genetic expression in the cell body. This is especially true with the mobile trk receptors, as they are cytoplasmic receptors that can act retroactively to the cell body and change the production of neurotransmitters, receptors, or even genetic expression of these proteins (French, Humby, Horner, Sofroniew, & Rattray, 1999; Molnar et al., 1998; Ward & Hagg, 2000).

Binding of the trk A receptor to NGF may work on acetylcholinergic cell bodies located in the septal region that send projections into the hippocampus, and change acetylcholinergic function in the hippocampus (Cellerina, 1996). The fact that olanzapine alleviates the decrease in NGF of hippocampal neurons may be very important as to the mechanism through which olanzapine works to alleviate cognitive deficits in dopamine D_2-supersensitized rats. Changes in NGF content in the hippocampus may change the way cholinergic neurons communicate and in essence, change cognitive behavioral performance in the MWT. Clearly, this is speculative, but future studies will analyze changes in genetic expression of NGF, BDNF, and ChAT in the hippocampus to analyze whether these changes in NGF may lead to permanent changes in hippocampal neurons, suggesting that olanzapine has neuroprotective properties.

Gender Differences in D_2 Supersensitization

A recent study from our laboratory showed that gender differences were observed in yawning behavior. In the present study, Female-Quinpirole-Saline animals displayed a 4 fold

increase in yawning compared to Female-Quinpirole-Olanzapine, Female-Saline-Olanzapine, and Female-Saline-Saline. In males, this difference was a 5- 6-fold increase in Male-Quinpirole-Saline animals compared to Male-Quinpirole-Olanzapine, Male-Saline-Olanzapine, and Male-Saline-Saline animals.

One potential explanation for this difference in yawning behavior may be related to function of the dopamine D_2 receptor, and several studies have reported gender differences in dopamine function (Becker, Molenda, & Hummer, 2001; Dluzen & McDermott, 2004; Murray et al., 2003). For example, when a female rat is in estrous, the dopamine system has been shown to be maximally stimulated by psychostimulants such as amphetamine and cocaine. On the other hand, when the female is in diestrous, the dopamine stimulation is muted, so there appears to be a strong influence of estrogen on the response of the dopamine system to drug treatment.   It appears that dopamine function in females may be highly related to the fluctuation of ovarian hormones during the estrous cycle in females. Goldstein et al. (2002) conducted a study to examine symptoms in male and female schizophrenic patients and their response to olanzapine and haloperidol. It was shown that for the 6-week trial, females treated with olanzapine demonstrated a significant decrease in overall symptoms than any other group. Furthermore, pre-menopausal women had a significantly better response to treatment as compared to post-menopausal women. These results demonstrate the role of dopamine in schizophrenia as shown by the ability of olanzapine to enhance functioning in females versus males.

In rats, Schindler and Carmona (2002) have reported gender differences in response to dopamine agonists and antagonists in locomotor activity. Animals were treated with cocaine, the D2 agonist GBR 12909, or D_1 agonist SKF 82958 after a habituation phase of the apparatus and then tested on the locomotor activity chamber for 30 minutes. Results showed that although both

sexes treated with dopamine agonists demonstrated an overall increase in activity compared to saline animals, female rats demonstrated greater increases in activity than male rats. These results show that females were more sensitive to the effects of these drugs as reflected in activity. In general, female animals demonstrated a significantly greater increase in activity in response to amphetamine (Frantz & Van Hartesveldt, 1999). However, our laboratory has recently shown that males may demonstrate an increase in sensitivity to the cognitive deficits produced by the D_2 antagonist, eticlopride (Brown et al., in press). Therefore, although females may demonstrate an increased sensitivity to the locomotor activating effects of particular dopaminergic agonists, males demonstrate increased sensitivity on other behaviors, such as yawning behavior and cognition. This appears to show that the relationship of the dopamine D_2 receptor to behavior is both gender and task specific, and differences in receptor sensitivity influenced by sex hormones and may be behaviorally manifested in different ways.

Gender Differences in Cognitive Deficits. In the current study, gender differences in MWT performance depended on the version of the task tested. Although there was a slight gender difference in acquisition latency, there were no other gender differences on the place version of the MWT. However, there were robust significant gender differences in animals tested on the Matching-to-Place Version of the MWT. The female-Q-O group showed improved performance compared to all other female groups. This was contrary to the hypothesis, as we predicted that Group Q-S would demonstrate a significant deficit relative to all other groups.

Possibly the most surprising result regarding gender differences and MWT performance in the present study was that the control group, female Group S-S, did not demonstrate learning of the match-to-place task. However, this is similar to a result from a previous study (Brown, et al., 2004a) that demonstrated 25-29-day old control females failed to learn this version of the

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MWT. The underlying mechanism of the observed improvement is unknown; however, it may be that olanzapine is improving performance through its effects on the dopaminergic system, hence the differences displayed among the females.

There have been studies that have shown deficits in female rats tested on the MWT. For example, Roof and Stein (1999) trained rats to locate a randomly placed platform, but were released from the same position. In this test, no gender differences were observed. However, in a second test, the release point was changed between trials and males performed superior to females. Males and females may adapt different learning techniques such as the use of different spatial cues. Also, Kolb and Cioe (1996) performed medial frontal cortex lesions on animals and then tested them on the Morris Water Task and a landmark task. It was found that the animals used a single spatial cue on the landmark task and found no gender differences in the brain-lesioned animals. However, on the more complex Morris Water Task, brain-lesioned females demonstrated a significant deficit to males, again suggesting males and females differ on spatial functioning.

Applications for the Current Study

Because schizophrenia is such a prevalent disease affecting approximately 1% of the population, research is needed to identify the underlying mechanisms responsible for the disorder as well as to test treatments for the disease. Research in pharmacological treatments costs approximately 2 billion dollars per year for a pharmaceutical company (http://www.lilly.com/about/highlights.html). To decrease the cost of the process, more effective models need to be use.

There are two primary applications of the results of this study. First, we have concluded that olanzapine alleviates cognitive deficits produced by increases in dopamine D_2 sensitivity. At

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the present time, olanzapine is the only atypical antipsychotic that has been shown to alleviate cognitive deficits in human schizophrenics (Bilder et al., 2002; Smith, Infante, Singh, & Khandat, 2001). Second, we have shown that chronic olanzapine treatment alleviates the decrease of NGF produced by neonatal quinpirole treatment in the hippocampus although not in the frontal cortex. This may suggest that chronic olanzapine treatment may have neuroprotective properties specific to the hippocampus. If this is the case, then olanzapine may be able to reverse exert its effects on behavior through its effects on neurotrophic factors, which appear to be related to synaptic growth and maintenance in the hippocampal region. Future studies will need to analyze the effects of olanzapine over longer treatment periods; however, the present research offers a clinical application of this drug not before observed.

On the other hand, chronic olanzapine treatment did not produce any significant effects on NGF or BDNF in controls, contradicting very recent findings by Parikh, Terry, Khan, and Mahadick (2004) that have shown chronic olanzapine treatment decreased NGF in the hippocampus. However, the brain tissue in this study was analyzed after an eight-day washout, and the tissue was analyzed one day after drug treatment. Additionally, slightly different drug treatment paradigms were used in the present study as compared to that by Parikh et al. which used significantly higher doses (5 mg/kg and 10 mg/kg).

In another study conducted by Parikh, Khan, and Mahadick (2004), it was shown that the typical antipsychotic haloperidol produced changes in the expression of BDNF; however, after treatment with olanzapine, BDNF and TrkB receptors were restored in the hippocampus. This finding is an agreement with the present study in that olanzapine reversed significant changes in neurotrophic factors, further supporting findings which have shown olanzapine may be neuroprotective.

Another important application of the current study is the support it provides for the dopamine supersensitization model as a model of schizophrenia. It is believed that this model may be useful to researchers in improving upon this drug and development of new medications for the treatment of schizophrenia. Research is currently being conducted to improve on current drug interventions that relate to the dopamine supersensitization model may prove effective to test these drugs to demonstrate their medical application.

Limitations of the Current Study and Suggestions for Future Research

Although the current study provides many insights to the causes and treatments for schizophrenia, additional research is still needed to better explain these results. The current study showed that olanzapine alleviates both cognitive deficits and D_2 receptor supersensitization in a rodent model of dopamine dysfunction. It was also able to provide insight to a few of the neurochemical effects of schizophrenia and antipsychotics. However, in order for researchers to provide further answers into diseases such as schizophrenia using this model, more in depth procedures must be used.

One area that was unable to be completed in the current study was the examination of acetylcholine in the brain. However, a preliminary analysis of optical density of the reading for ChAT demonstrated a significant decrease of hippocampal ChAT in rats neonatally treated with quinpirole that was not alleviated by olanzapine. An important effect of D_2 supersensitivity is the modulation of the acetylcholinergic system in the hippocampus. Studies have shown that activation of the D_2 receptor produces a significant decrease in acetylcholine in the dorsal hippocampus, and these results are in agreement with these past findings. However, olanzapine has been shown to increase acetylcholinergic tone in this same region (Shirazi-Southall et al., 2002), and this does not appear to be replicated in this study. These results should be treated as a

preliminary conclusion, as more analysis needs to be done, and not all brain tissue has yet been analyzed. Further, many studies have shown that olanzapine has the ability to eliminate cognitive deficits (Sharma, Hughes, Soni, and Kumari, 2003; Wolff & Leander 2003), but the mechanism through which olanzapine is acting is not known.

We have previously shown that neonatal quinpirole treatment produces a significant decrease in acetylcholinergic tone in the hippocampus. Nicotine, which has been shown to alleviate cognitive impairment, has been shown to partially alleviate this loss of acetylcholinergic tone (Brown et al., 2004b). One of the most important neurotransmitter systems in the brain in cognitive function is the acetylcholinergic system of the hippocampus cholinergic system. Loss of acetylcholinergic tone, especially in the hippocampus, has been hypothesized to play an important role in cognitive impairment (Scarr, Copolov, & Dean, 2001; Perry, Walker, Grace, & Perry, 1999).

As previously discussed, one marker of acetylcholine function is the nicotinic acetylcholine receptor (nAChRs). These receptors are found in many brain areas important in cognitive function such as the hippocampus and frontal cortex (Levin & Simon, 1998; Mihailescu & Drucker-Colin 2000). Studies examining nicotine use in schizophrenics indicate these patients demonstrate improvement in cognitive functioning in tasks requiring attention, working memory, short-term memory and recognition memory (Cattapan-Ludewig, Ludewig, Jaquenoud, Etzensberger, & Hasler, in press; Sacco, Bannon, & George, 2004), and nicotine increases nicotinic receptors throughout the brain. To improve cognitive functioning in schizophrenia patients, the role of acetylcholine must be studied further.

One important caveat is the role genetic factors play in the predisposition of schizophrenia. Family pedigree studies have shown that schizophrenia is more common among

relatives of people with schizophrenia and the more closely related the relatives are, the more likely the person is to develop schizophrenia (Comer, 2001). Studies have shown that schizophrenic patients may have gene deficits on chromosomes 5, 6, 8, 9, 10, 11, 13, 18, 19, and 22 (Kendler et al., 2000; Pulver, 2000). These genetic factors may lead to the development of schizophrenia through biochemical abnormalities, such as dopamine dysfunction, and abnormal brain structures, such as enlarged ventricles. One method to study these changes through the use of the dopamine supersensitization model is the polymerase chain reaction (PCR), and to analyze whether particular proteins may be related to chromosomal abnormalities are also affected.

In recent findings from our laboratory, we have shown that neonatal quinpirole treatment results in increases of genetic expression of the alpha$_7$ nicotinic receptor subtype in the hippocampus. Additionally, past studies by Tizabi et al. (1999) have shown that the alpha$_7$ receptor is increased in binding in the same region using the autoradiographic technique. This is contrary to what has been found in human schizophrenics post-mortem, which have shown that the alpha$_7$ nicotinic receptor is decreased in expression. Regarding our model, this may be important in explain some of the behavioral deficits we have reported as alpha$_7$ receptors are primarily presynaptic, and when bound, increase the release of neurotransmitter. Alpha$_7$ receptor subtypes have been found on dopaminergic, serotinergic, glutmatergic, and acetylcholinergic neurons. Therefore, rats neonatally treated with quinpirole may have increased neurotransmission across several neurotransmitter systems, which may lead to cognitive deficits observed in the present study. More research must be done to localize what other protein changes may exist elsewhere in the brain in dopamine D_2-primed rats.

While the current model has demonstrated some similarities to that of human studies, it does not necessarily maintain characteristics that are found in human patients. For example,

human studies have shown that, when examined by Zaidel, Esiri, and Harrison (1997), the hippocampi of schizophrenic patients appear to be smaller than controls. We have not tested whether the hippocampi are smaller in D_2-primed rats, and volumetric studies need to be done. However, in brain dissection, the weights of the hippocampi of D_2-primed rats do not significantly differ from controls. The lack of a difference of size of hippocampus may be a shortcoming of the current model. However, results have shown that neonatal quinpirole treatment results in a significant decrease in hippocampal NGF at three different ages (P30, P75, and P110) suggesting a loss of synaptic connectivity and maintenance of connections in this region (Brown et al., 2004a; 2004b; unpublished data). Although hippocampal volume has not been measured in these animals, the loss of NGF at least suggests changes in this brain region that may be consistent with that of schizophrenics.

With the elimination of dopamine D_2 supersensitivity when the appropriate antipsychotics are prescribed to schizophrenia patients, these patients may have more reliable treatment alternatives. The changes in the dopamine system may mean that the cognitive deficits may be reduced or completely eliminated by olanzapine, allowing these patients to lead more normal lives than without treatment. Additionally, neurochemically these patients may be shifted more towards normal brain functioning. Schizophrenics have also demonstrated an increased propensity to abuse psychostimulants (Le Duc & Mittleman, 1995), which also may be due to a hyperactive dopaminergic system, based on the fact that this neurotransmitter system plays a primary role in drug abuse.

In conclusion, schizophrenia is a complex disease both behaviorally and neurochemically, and new treatments must be identified to help treat this disorder. The current model, despite a few weaknesses, appears to have application to not only build a defense for the

cause of the disease but also to demonstrate the effectiveness of the current methods of treatment available to schizophrenia patients today. In the future, studies will evolve from this model to make a stronger science and further the study of the schizophrenia and other disease treatments and alleviations.

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