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An Analysis of Nicotine Exacerbation of Reductions in PPI in a Rodent Model of Schizophrenia.

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by

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ABSTRACT

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Prepulse inhibition (PPI) is an operational measure of sensorimotor gating and is known to be reduced when the dopamine D_2 receptor is activated. We used a rodent model of psychosis in which increases in dopamine D_2 receptor sensitivity are produced through neonatal quinpirole (a dopamine D_2 / D_3 agonist) treatment to rats. Rats were administered quinpirole (1mg/kg) or saline from postnatal day (P) 1-21. Rats were raised to adulthood and tested on PPI. Results showed that neonatal quinpirole treatment produced a significant reduction in PPI, and nicotine exacerbated this reduction. This reduction was partially blocked by the nicotinic antagonist mecamylamine. Brain tissue was analyzed for regulators of G-protein signaling (RGS) and results showed that neonatal quinpirole significantly decreased RGS9, but increased RGS17 as compared to controls. These results appear to indicate that the G-protein couples more efficiently to the D_2 receptor, and nicotine exacerbates PPI deficits in D_2 receptor-primed rats.

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CHAPTER 1

INTRODUCTION

Schizophrenia

Schizophrenia is a complicated disease that has puzzled scientists since its first classification by Emil Kraepelin in 1896. The disease, which he named *dementia praecox,* was characterized by an early onset and was followed by progressive deterioration of the mind. In 1911, Eugen Bleuler introduced the term schizophrenia, meaning "*split mind.*" Bleuler believed that schizophrenia was a split between the emotional and intellectual aspects of the person. He also concluded that the four main symptoms of schizophrenia were impaired association of ideas, disorder affectivity, marked ambivalence, and autism (Stotz-Ingenlath, 2000). Today, approximately one percent of the general population is diagnosed with schizophrenia. This disease has been difficult to study because there are many different aspects and characteristics of the disease. Of those patients diagnosed with schizophrenia, it would be difficult to find one common behavioral characteristic in every patient (Andreasen, 1999). The DSM-IV has six diagnostic criteria, with many different subtypes within those six criteria. For an individual to be diagnosed with schizophrenia, a person must display two or more of the characteristic symptoms for a 6-month period. It is rare to find a person who displays all of the symptoms of the DSM-IV symptomology for schizophrenia (Berenbaum & Oltmanns, 1992). However, an extensive body of research has shown that humans with schizophrenia show a significant deficit in their sensorimotor gating of auditory stimuli (Braff et al., 1978; Swerdlow et al., 2005).

Disruptions in cognition and processing of sensory information are two of the characteristics of schizophrenia. Since the sensory and attention deficits were clinically observed in humans with schizophrenia by Bleuler and Zinken in 1952 (as cited in Geyer, Krebs-Thomson, Braff, &

Swerdlow, 2001), researchers have been attempting to determine the specific brain regions and underlying mechanisms responsible for the behavioral disruptions known to be present in schizophrenia. Researchers have also explored how different drugs interact with specific mechanisms and brain regions that are known to be involved in the disease (2001). The experiments described will focus on a rodent model of schizophrenia and will analyze auditory sensorimotor gating deficits and how nicotine, a commonly abused drug in the population of humans with schizophrenia, affects auditory sensorimotor gating deficits. Finally, we will analyze genetic mechanisms of these behavioral impairments through analysis of genetic transcripts known to regulate G-proteins, which are involved in dopaminergic cell signaling.

Sensory Disruption in Schizophrenia

McGhie and Chapman (1961) were the first to find that early onset of humans with schizophrenia had difficulties with sensory perception and attention. One way in which perception and attention is analyzed in humans with schizophrenia is through sensorimotor gating. Sensorimotor gating is the ability of neurobiological networks to transmit selectively incoming auditory stimulus to the brain and by filtering out irrelevant stimuli (Birbaumer & Schmidt, 1996). This mechanism protects the brain from too much information entering the sensory system and possibly overloading sensory systems, which would produce deficits in attention and sensory perception. In humans with schizophrenia, one of the more prevalent findings in this research literature is that auditory sensorimotor gating is not functioning properly, therefore, causing sensory overload in the auditory sensory system (Geyer et al., 2001). Interestingly, auditory sensorimotor gating deficits have not only been shown in humans with schizophrenia but also in the relatives of humans with schizophrenia, suggesting a hereditary basis of this type of sensory impairment (Ringel, Heidrich, Jacob, & Fallgatter, 2004). Ojeda et

al. (2002) concluded that although specific behavioral deficits in schizophrenia improve with treatment, auditory sensorimotor gating deficits remain persistent in humans with schizophrenia. Therefore, it has been suggested that auditory sensorimotor gating deficits are a fundamental characteristic of the disease (Geyer et al., 2001).

The deficit in auditory sensorimotor gating may be related to the dysfunction in the dopaminergic system known to be present in schizophrenia (Leonard et al., 2000) because other diseases that are related to dopaminergic dysfunction such as Huntington's disease, Parkinson's disease, Tourette's syndrome, and obsessive-compulsive disorder are also all known to produce deficits in auditory sensorimotor gating (Geyer et al., 2001; O'Neill, Rieger, Kem, & Stevens, 2003; Swerdlow et al., 2002).

Animal Models of Psychosis

One of the most common ways of researching a disease is to model the same aspects of the disorder in animals. Animal models may be used to study the cause of a disease or to compare treatments for a particular disease but may not be able to predict the progression of the disease over a lifetime. However, animal models may be useful for development and discovery of new and more effective treatments for neurological disorders (Woodruff & Baisden, 1994). In many cases, animal models of neurological disease and dysfunction are used to model one aspect of the disorder, whether it be behavioral, neurochemical, or neuropathological. This can be extremely useful because it can be informative about the contribution of this behavioral or neurochemical abnormality to the disease or disorder, although the entire disorder is not modeled in the animal. McKinney and Bunney (1969) proposed three different criteria for validating animal models of human psychopathology. The three criteria are similarity of inducing conditions, similarity of behavioral states, and similarity of common mechanisms in an animal model. They concluded

that the higher number of criteria the model has means that the model has increased validity. Regardless, a rodent model may still be informative even without meeting all the criteria (Woodruff & Baisden, 1994). Therefore, an animal model of psychopathology could be used for examination of the disease even though it did not contain all aspects of the disease.

The importance of animal research is evident in the ability to generalize findings using animal subjects to the human health condition. Generalization of research on schizophrenia from animal to human is possible through using similar behavioral tasks with specific task demands, testing parameters, and mechanisms of the behavioral deficits involved in the disorder. Gage, Bjorklund, Isacson, and Brundin (1985) defined animal models of diseases as correlative, analogous, or homologous. A correlative animal model is one in which the animal would respond in a similar way to a drug treatment. When dealing with neurological disorders, an analogous animal model is used in which one or more anatomical regions of the central nervous system are damaged similar to those in the human disease. A homologous animal model is when the same disease is found in the animal as in the human, but it is often difficult to find with most diseases. One of the advantages of using auditory sensorimotor gating is that the testing parameters used on human patients can easily be modeled in rats.

Rodent Models of Schizophrenia

The primary animal models of schizophrenia that have been used are the neonatal hippocampus excitotoxic lesion model, the phencyclidine (PCP) model, the amphetamine model, and the dopamine agonist model. The most prolific rodent model of schizophrenia that has been studied is the neonatal hippocampal lesion model, which has been used in both rats and monkeys. One reason this model has been so heavily used is because the functional and structural changes produced by neonatal hippocampal lesions in both the rodent and primate

hippocampus are similar to the neuropathology found in humans with schizophrenia. The goal of this model is to damage regions of the hippocampus that project to the prefrontal cortex, primarily the ventral hippocampus (VH) and ventral subiculum (Lipska, 2004). In rats, neonatal VH lesions produce relatively small behavioral changes in young adolescents (postnatal day [P] 35); however, increases in behavioral changes are observed as they reach adolescence and adulthood. Younger adolescents VH lesioned rats are less social than controls but otherwise do not demonstrate any other behavioral deficits. However, later in adults, VH lesioned rats display behaviors thought to be linked to an increase in mesolimbic and nigrostrial dopamine transmission, resulting in motor hyperresponsiveness to stress and stimulants and enhanced stereotypic motor behavior (Lipska). They exhibit enhanced sensitivity to glutamate antagonists such as MK-801 (dizoclipine) and PCP. In addition, they demonstrate deficits in sensorimotor gating, latent inhibition, social behaviors, and working memory (Chambers, Moore, McEvoy, & Levin, 1996; Flores, Barbeau, Quirion, & Srivastava, 1996; Lipska, Jaskiw, & Weinberger, 1993; Wan, Hartman, & Corbett, 1998). Appearance of these abnormalities in later adolescence and adulthood highly parallels the experiences of human patients with schizophrenia, who generally do not experience deficits until adolescence (Lipska).

Neonatal Hippocampal Lesion Model. One problem with the neonatal hippocampal lesion model is that to successfully lesion an area of the hippocampus a drug such as *N*-methyl-D-aspartic acid (NMDA) is injected into the brain causing excitotoxicity. In excitotoxicity, high levels of calcium ions enter the cell increasing the number of enzymes; these enzymes go on to damage the cell structures, therefore, causing apoptosis of the cell. Although a valuable model of schizophrenia, actual apoptosis is not found in humans with schizophrenia (McGlashan, 2006). However, there are physiological abnormalities in the brain of humans with schizophrenia such

as an increase in ventricles (Antonova, Sharma, Morris, & Kumari, 2004). While there are many anatomical similarities between the VH lesioned rats and those persons who have schizophrenia, there are physiological differences. For example, in the VH lesioned rat's prefrontal cortex there are increases in synaptic densities, number of branches, and dendretic lengths. However, human patient with schizophrenia have decreases in dendritic connectivity because of a decreases in synaptic densities, number of branches, and dendretic lengths. The neonatal VH model can be hypothesized as a partial analogous rodent model of schizophrenia from which valuable information has been obtained.

PCP Model. Phencyclidine (PCP) was first developed in 1956 and was used as an anesthetic in humans before being abandoned because of serious psychiatric reactions. These serious reactions included agitation, excitement, delirium, disorientation, and hallucinatory phenomena. These reactions mimic several aspects of schizophrenic symptomology in normal volunteers. Sams-Dodd (1999), in a series of studies, administered PCP to rats to attempt to replicate both positive and negative symptoms of schizophrenia. In a social interaction test, it was concluded that PCP dose-dependently induces stereotyped motor behavior and social withdrawal, which positively correlates with both negative and positive symptoms of schizophrenia. PCP interacts in the brain by blocking the PCP binding site located on the glutametergic NMDA receptor, acting as an inverse agonist. In this animal model, PCP causes a hypoactivity of the NMDA receptor that is also found in human schizophrenics. The effects of PCP were selectively reduced with antipsychotic drug treatment; however, drugs lacking antipsychotic effects have not been shown to alleviate PCP-induced behaviors (Sams-Dodd).

Amphetamine Model. The rat amphetamine (AMPH) induced model of schizophrenia depends on the ability of a high dose of amphetamine or withdrawal from amphetamine to causes

increased sensitization of dopamine receptors in the brain. The decision to administer AMPH to rats to produce a rodent model of schizophrenia was concluded after the reaction of AMPH in humans. Furthermore, humans who were given AMPH displayed persistent movement patterns, sudden outbursts of aggression and violence, and paranoid delusions (Julien, 2003). Therefore, in rats administration of AMPH generally causes increased psychomotor activity. These behaviors appear to be mediated by dopamine receptors in the mesolimbic dopamine system. These behaviors appear to involve dopaminergic activity in the caudate nucleus and putamen of the basal ganglia (Julien). In humans, during withdrawal from amphetamines persistent paranoid symptoms and disruptions in latent inhibition (LI) have been reported.

LI is a behavioral task in which a participant or animal is tested on responding to a conditioned stimulus (CS) that was repeatedly presented without reinforcement prior to the CS-unconditioned stimulus (US) pairing. In the AMPH induced animal model of schizophrenia, disrupted LI mediated by dopamine produces and exacerbates increases in locomotor activity, which can be reversed by typical and atypical antipsychotic drugs. LI disruption is also found in humans with schizophrenia (Zuckerman, Rehavi, Nachman, & Weiner, 2003). This LI disruption animal model produced by AMPH with positive symptoms is considered to have face, construct, and predictive validity (Zuckerman et al.).

Dopamine D_2 Receptor Priming Model. Finally, the dopamine (DA) agonist animal model of schizophrenia has been used because it is considered to be both a correlative and analogous model of the disease. One proposed cause for schizophrenia is an increase in dopamine activity at the dopamine receptor. Many animal models of schizophrenia have attempted to create dopamine hyperactivity by acute administrations of pharmacological agents that produce dramatic increases in dopamine release such as phencyclidine or cocaine (Lacroix, Broersen,

Feldon, & Weirner, 2000; Tilson & Rech, 1973). Although useful information is gained about the acute robust increase of dopamine in the brain and behavior, these models give little information about the long-term increases in dopamine activity, which is observed in the disease.

Specific to the dopamine system, increased activation of the D_2 receptor has been shown to play a major role in abnormal behaviors observed in schizophrenia, also drugs that enhance dopaminergic receptor function produce similar symptomology in humans (Castaneda, Becker, & Robinson, 1998; Davis, Kahn, Ko, & Davidson, 1991; Kokkindis & Anisman, 1980). Furthermore, effective antipsychotic drugs such as risperidone and olanzapine are antagonists of the D_2 receptor. Research from Kostrzewa, Kalbfeisch, Perry, and Fuller (1994) has demonstrated that quinpirole, a dopamine D_2/D_3 agonist, administered to rats from postnatal day (P) 1-11, 1-21, or 21-35 produces priming of the D_2 receptor that persists throughout an animal's lifetime. It is recognized that schizophrenia appears to be more than an abnormality in dopamine system functions, as other neurotransmitter systems have been implicated in this disease. However, several studies have shown that dopamine hyperactivity modulates other neutoransmitters including serotonin, norepinephrine, and acetylcholine as well as neurohormonal activity via the hypothalamic-pituitary axis (Cotter & Pariante, 2002; Crook, Tomaskovic-Cook, Copolov, & Dean, 2000; Leonard et al., 2002). This suggests that hyperactivity of the dopamine system produces modulation of other neurotransmitter or neurohormone systems that may also play a role in the behavioral deficits of the disease.

Quinpirole induced long-term D_2 receptor priming can be considered a valid animal model of schizophrenia because of the several consistencies found between the effects of neonatal quinpirole and data from the human schizophrenia literature:

1) Amphetamine administration to adult rats neonatally treated with quinpirole produce a robust increase in release of dopamine in the striatum (Nowak, Ryszard, & Kostrzewa, 2001). Also, studies using MRI and positron emission tomography (SPECT) imaging have shown that amphetamine administration produces a large increase in dopamine release in the striatum of humans with schizophrenia (Lavalaye, Booij, Linszen, Reneman, & Van Royen, 2001; Soares & Innis, 1999).

2) Neonatal quinpirole treatments have been shown to produce long-term cognitive impairments (Brown, Gass, & Kostrzewa, 2002). It has been well documented that mild to severe cognitive impairments are present in humans with schizophrenia; it has been suggested that cognitive impairment is a core feature of the disorder (Adler et al., 2000; Elvevag & Goldberg, 2002).

3) 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 produced by neonatal quinpirole treatment in rats. Brown et al. (2004) have shown that chronic olanzapine treatment given twice daily in adulthood alleviates cognitive deficits produced by neonatal quinpirole treatment.

4) Neonatal quinpirole treatments have been shown to produce neurochemical abnormalities in adulthood that are similar to observations made in humans with schizophrenia. Results from this laboratory have shown that neonatal quinopirole treatment produced a 36% decrease in choline acetyltransferase (ChAT) and significantly decreased nerve growth factor (NGF) expression in the hippocampus compared to saline controls in both early postweaning and adult rats (2004).

5) Neonatal quinpirole treatments have been shown to produce deficits in auditory sensorimotor gating using prepulse inhibition (PPI), different prepulse auditory intensities (73, 76, and 82 dB), and different interstimulus intervals between the prepulse and pulse (50, 100, and 150ms). Previous studies from this laboratory have shown that adult rats that received neonatal quinpirole treatment demonstrated PPI deficits as compared to controls. As previously mentioned, sensorimotor gating can be measured in humans and deficits in PPI, prepulse inhibition of perceived stimulus intensity (PSIPSI), and auditory P50 are considered hallmark characteristics of schizophrenia (Swerdlow et al., 2002).

Nowak et al. (2001) have provided both the ability of quinpirole to induce long-term sensitization and insight into its mechanism of action. Long-term sensitization to DA is hypothesized to cause psychopathologies such as psychosis, mania, post-traumatic stress disorder, panic disorder, and addiction (Einat & Szechtman, 1993). Nowak et al. (2001) produced DA "priming" by administering neonatal treatments of quinpirole (50 μg/kg/day) to rats, from the 1st to 11th days after birth. DA sensititzation was confirmed behaviorally by an increase in quinpirole-induced yawning in adulthood. They concluded that AMPH (1.0 mg/kg, ip) acutely induced a five-fold increase in DA in the neostriatal in vivo microdialystate of those quinpirole- primed rats. It is believed that the behavioral sensitization to AMPH is because of subsensitization of the ventral tegmental area (VTA) (A10) presysnaptic D_2 autoreceptors, AMPH-induced enhancement of DA release, up-regulation of the DA transporter, and supersensitization of postsynaptic D_2 receptors (2001). These are all areas that are primed by neonatal administration of quinpirole then activated by adulthood AMPH injections. The proposed study is an example of how quinpirole induced DA sensitization can increase dopamine levels when stimulants such as APMH are introduced in adulthood. These stimulant induced

dopamine increases can be generalized to human psychopathologies, which are believed to also have a sensitized dopamine system. A sensitized dopamine system would be a system that would have an increase in reaction to dopamine, specifically at the postsynaptic dopamine receptor (Kostrzewa et al.,1994).

Sensorimotor Gating Testing

Three different types of behavioral tasks have been used to test sensorimotor gating: Prepulse inhibition (PPI), Prepulse inhibition of perceived stimulus intensity (PPIPSI), and the P50 auditory evoked potential conditioning testing paradigm (Braff et al., 2001; Mickey & Dalack, 2005; Swerdlow et al., 2005).

Prepulse Inhibition

The prepulse inhibition (PPI) task has been performed in both humans and animals using very similar parameters. When used for auditory sensorimotor gating, PPI is a task in which the subject is given a startling auditory stimulus (115-120dB) that is preceded by a weaker intensity auditory stimulus (73-82dB). The dependent measure is the subject's ability to inhibit a startle response to the startling stimulus when the prepulse precedes the startling stimulus. A startle response is defined as any movement that happens as a result of the auditory stimulus. This inability has been determined to be an adequate measurement of sensorimotor gating deficit because the task involves the inhibition of the startle response (Swedlow et al., 2001). PPI deficits, such as the inability to inhibit the startle response, have been associated with thought disorder, distractibility, positive and negative symptoms, and early onset of schizophrenia (Braff et al., 2001). Braff et al.(1978) were among the first researchers to demonstrate that humans with schizophrenia have a deficit in PPI when compared to controls. Therefore, PPI is both an animal and human behavioral task of sensorimotor gating.

PPIPSI and the Auditory P50

Helen Peak (1939) first developed "pre-pulse inhibition of perceived stimulus intensity" (PPIPSI) for testing auditory gating deficits (Swerdlow et al., 2002). PPIPSI is a direct report of the perceived intensity of a second stimulus in the presence and absence of a pre-stimulus. Under correct conditions, participants report that they perceive an intense abrupt stimulus e.g. a 118 dB noise burst, a 40-psi air puff, or a 170 V cutaneous shock to be less intense if it is preceded by a weak prepulse (2002). Humans with schizophrenia will react to the second shock or pulse to be at its actual dB or V level, therefore, not factoring the first prepulse as does a control (2002). The PPIPSI testing has not been drastically modified since its original conception in 1939. Whereas the PPIPSI test for sensory gating is self-assessment, the P50 paradigm is a more precise physiological assessment of the sensory gating deficits.

The dual click P50 paradigm is an electrophysiological technique used to examine gating mechanisms (Adler et al., 1982). The P50 is mediated by the first (conditioning) stimulus (S1), which activates an inhibitory system that reduces the response to the second (test) stimulus (S2). Therefore, the magnitudes of these electrical signals are measured by the P50 event- related potential (ERP). The ERP is a positive-going auditory component that appears approximately 50 ms after presentation of the stimulus. The ERP is measured by using an electroencephalograph (EEG) in humans. In a healthy participant, Adler et al. (1982) found a significant reduction in the ERP after the second test stimulus. Similar results corroborated the evidence for high suppression in healthy participants and a lack of suppression of the ERP in humans with schizophrenia (Waldo, Myles-Worsley, Madison, Byerley, & Freedman, 1995). Therefore, the lack of the inhibitory second ERP signal in the P50 paradigm has been suggested to result from too much information being sent to the somatosensory cortex in patients with schizophrenia, thus

strengthening the evidence for the sensory gating deficits found in schizophrenic patients. The reduced sensory gating in the dual click paradigm seems to be a phenotypic marker for a genetic deficit because the deficit was also found in one half of the first-degree relatives of humans with schizophrenia (Waldo et al., 1995). The P50 dual click paradigm has also been used in rodent models of the schizophrenia where the dopamine system is increased in activity. Recordings from electrodes placed the in the animal's brain have shown similar results because of the lack of suppression of the ERP after the second stimuli (Geyer et al., 2001).

Brain Involvement in Prepulse Inhbition

Brain Circuitry Mediating PPI

PPI functioning has been shown to be mediated by complex circuitry in the brain. Recent research suggests that PPI is regulated by both sequential and parallel neural connections between the limbic cortex (including temporal cortex and medial prefrontal cortex), the ventral striatum, the ventral pallidum, and the pontine tegmentum (Swerdlow et al., 2001). Swerdlow et al. (2002) determined the areas and pathways of the brain involved in PPI functioning by producing chemical or physical lesions in specific areas of the brain. The two main neural elements that control PPI can be divided into those that mediate PPI and those that regulate PPI. The brain circuitry that mediates PPI is activated by the prepulse and then transmits the neural consequences of the prepulse in such a way as to inhibit some of the neural and behavioral results of the startling pulse (2002). This mediating circuitry seems to be activated by the product of the velocity with which the prepulse or its neural consequences travel across the neural tissue, and the time period (prepulse interval) in which reflex inhibition is first observed. This time period seems to be 20 ms in the rat, depending on specific testing conditions (Ison, Taylor, Bowen, & Schwarzkopf, 1997; Swerdlow et al., 2001). Fendt, Li, and Yeomans (2001)

describe the "primary" mammalian acoustic startle response (ASR) circuit that mediates PPI as including several connections linking the auditory nerve, brain stem, and the spinal motor neurons.

Currently, there are no studies that provide convincing evidence for or against any one specific pathway that mediates PPI. However, there is evidence that whatever path is traveled by the prepulse signal, it is capable of inhibiting the startle by neural elements contained within the pons or brainstem (Davis & Gendelman, 1977). Therefore, the brain circuitry mediating PPI is involved with the uptake of the neural impulse of the prepulse and the subsequent startle to the prepulse.

Brain Circuitry Regulating PPI

The brain circuitry that regulates changes in PPI involves the mediating circuitry's impact of the prepulse on the pulse. This regulating circuitry can best be described as a tonic "thermostat" influence on the mediating circuitry. This "thermostat" can be adjusted by changes in the regulatory circuitry imparted by affective or attentional states, by pharmacological influences, or by neuropathological changes (Swerdlow et al., 2001). There is a substantial amount of information about the regulation of PPI by the forebrain circuitry; however, there is still disagreement in the precise regulating circuitry of PPI because so much of the brain is involved (Geyer et al., 2001; Soares & Innis, 1999; Swerdlow et al., 2001).

Changes in regulating PPI functioning are observable after experimental manipulation of three "limbic cortical" subregions in the rat: the hippocampus (HPC), the prefrontal cortex (PFC), and the amygdala. Activity in these limbic cortical areas regulates PPI behavior in part because of their subcortical projections to the nucleus accumbens (NAC). Lesions of these brain areas disrupt PPI and may be relevant to the neonatal hippocampal lesion model of schizophrenia

in that there is pathology amongst these connections in schizophrenia. Therefore, the functioning of these areas determines the animal's ability or inability to inhibit the startle response to the pulse in PPI task.

Brain Areas in PPI

Hippocampus. Findings have shown that PPI is significantly reduced in patients with temporal lobe epilepsy with psychosis compared to temporal epiletpic patients without psychosis (Morton et al., 1994). The hippocampus is a structure in the temperoral lobe. In animal studies, carbachol, a cholinergic agonist, infusion into the hippocampus disrupts PPI of both acoustic and tactile startle suggesting that the hippocampus modulation of PPI is not modality-specific (Caine et al., 1991). Along with the hippocampal cholinergic substrate, hippocampal glutamatergic activity appears to regulate PPI; infusion of the glutamate agonist NMDA into the ventral hippocampus profoundly reduces PPI in rats, and this effect is reversed by co-infusion of the NMDA agonist AP5 (Wan et al., 1998). The hippocampal regions that affect PPI performance through the gluatamatergic system appear to be more localized to the regions of the ventral subiculum and entorhinal cortex (Cain, Geyer, & Swerdlow, 1991). The areas of the hippocampus that affect PPI performance through the cholinergic system seem to include the dentate gyrus, ventral subiculum, and regions of the hippocampus proper.

Swerdlow et al. (2001) suggest that hippocampal damage can modify the PPI-disruptive effects of dopaminergic activation. Specifically, ibotenic acid lesions of the ventral hippocampus in adult rats result in the delayed development of "supersensitivity" to the PPI-disruptive effects of the DA agonist apomorphirne. When hippocampal lesions are made in 7-day-old rats, apomorphine-supersensitiviy is not evident until post-puberty, consistent with existing developmental models for the delayed emergence of the underlying pathophysiology of

schizophrenia (Weinberger, 1987). These findings suggest strong evidence for PPI functioning in the hippocampus and specific neurotransmitters in the hippocampus.

Prefrontal Cortex. The prefrontal cortex (PFC) in rats seems to regulate PPI in a manner that parallels the proposed role of reduced prefrontal cortex dopaminergic activity in schizophrenia (Csernansky, Murphy, & Faustman, 1993). PPI functioning is reduced by manipulations that decrease frontal cortex dopaminergic "tone," such as depletion of frontal cortex dopamine by infusion of 6-hydroxydopamine or intra-PFC infusion of D_1 or D_2 agonists (Ellenbroek, Budde, & Cools, 1996). Furthermore, it has been proposed that reduced prefrontal cortex dopamine transmission disrupts PPI via disinhibition of descending glutamatergic fibers that result in subcortical increases in DA transmission in the nucleus accumbens. Zavitsanou et al. (1999) concluded that the cognitive disturbances observed in schizophrenia are mediated by functional over activity of the mesolimbic DA projection system and/or functional under activity of the mesocortical DA system, which both involved the PFC area of the brain.

Amygdala. The amygdala was considered important in PPI functioning after Decker, Curzon, and Brioni, (1995) found that a large radio frequency lesion of the amygdala significantly reduced PPI in rats. This finding was confirmed by Wan and Swerdlow (1996) who demonstrated that small cell-specific quinolinic acid lesions of the basolateral amygdala potently reduced PPI. It has also been discovered that electrical kindling or intra-basolateral amygdala infusions of either picrotoxin or the NMDA receptor antagonist dizocilpine also disrupt PPI functioning in the amygdala (Fendt & Yeomans, 2001). This PPI disruption by intra-basolateral amygdala activation via either picrotoxin or dizocilpine appaear to be dopamine-dependent because the disruptions can be reversed by a high potency D_2 antagonist haloperidol. Dopamine reduces PPI performance via direct effects on the subcortical dopamine transmission [e.g. in the

nucleus accumbens core subregion, which is innervated by both medial prefrontal cortex and basolateral amygdala] (2001).

Nucleus Accumbens. The nucleus accumbens is directly involved in PPI functioning, possibly because of the projections received from the hippocampus, amygdala, and prefrontal cortex. Within the nucleus accubmens, this is a convergence of glutamatergic fibers from the hippocampus, medial prefrontal cortex, amygdala, and cingulate gyrus, and of dopaminergic fibers from cells in the ventral tegmentum (Swerdlow et al., 2001). The nucleus accumbens seems to be an integrated connection center connecting forebrain and limbic structures that control PPI. Also, several researchers have suggested that some of the effects of dopamine agonists on PPI may be mediated by increased dopamine activity in the nucleus accumbens (Swerdlow et al.). First, low doses of apomorphine that do not decrease PPI in control rats potently disrupt PPI in rats that are altered to have "supersensitive" DA receptors in the NAC (Swerdlow et al.). Second, DA increase disrupts PPI functioning, which can then be reversed by depletion of DA in the NAC. Finally, PPI is disrupted in rats by D_2 agonist quinpirole or DA into the NAC or anteromedial striatum (Swerdlow et al.). The effects of intral-NAC quinpirole, amphetamine or DA infusion on PPI are reversed by systemic treatment with D_2 antagonist (Swerdlow et al.). Therefore, one can conclude that NAC appears to be an important area for the DA agonist-induced loss of PPI in rats and the main "hub" for PPI projects in the brain.

Neurotransmitters Mediating Prepulse Inhibition

Although different neurotransmitters have been shown to mediate PPI performance, researchers have demonstrated that both dopamine and serotonin play primary roles in mediating a subject's performance in PPI testing (Geyer et al., 2001). Norepinephrine (NE) or noradrenaline-along with dopamine has come to be recognized as also playing a large role in

attention and focus, which are a large part of PPI functioning. Swerdlow et al. (2006) have concluded that PPI is regulated by both norepinephrine and dopamine substrates that are neurochemically separable. However, the majority of the literature on PPI testing and sensori-motor gating has focused on the role of dopamine in PPI.

Dopamine

In the brain, there are five types of dopamine receptors: D_1, D_2, D_3, D_4, and D_5. All dopamine receptors are G-protein coupled metabotropic receptors and can be excitatory or inhibitory to the post-synaptic neuron. Furthermore, the dopaminergic neurotransmitter consists of two families of receptors: the D_1 and the D_2. The D_1 and D_5 receptors are members of the D_1-like family, whereas the D_2, D_3, and D_4 receptors are members of the D_2-like family. A difference between the two families of receptors can be found in the mechanism of the G-protein. The activation of the D_1-like family receptors is coupled to increases in cAMP and is typically excitatory, whereas D_2-like activation reduces cAMP and is typically inhibitory (Zimmerberg & Weston, 2002). D_1 receptors also activate adenylyl cyclase (AC) via their coupling to G_s/G_{olf}, while D_2 recptors are $G_{i/o}$ linked and release $G_{\alpha i/o}$ and $G_{\beta\gamma}$ subunits (Bonci & Woodward, 2005). D_2 receptors also alter intracellular signaling through $G_{\beta\gamma}$ subunits, which can act at a number of intracellular targets (Neve, Seasmans, & Trantham-Davidson, 2004). The activity of the D_2 receptor is regulated by desensitization, where continuous agonist application results in phosphorylation of the D_2 receptor (e.g. by the G-protein receptor kinase GRK2), leading to uncoupling of receptors from G-protein activation and promotion of binding of arrestin and receptor internalization (Gainetdinov, Premont, Bohn, Lefkowitz, & Caron, 2004).

Researchers have determined that the D_2 receptor is perhaps the most sensitive to deficits in PPI by administering drugs that either act as agonists or antagonists at the D_2 receptor (Geyer et

al., 2001; Mansbach et al., 1988; Ralph, Paulus, & Geyer, 2001; Swerdlow et al., 2001). Results have shown that blockade of the D_2 receptor reduced PPI performance, and, interestingly, D_2 agonists have also been shown to disrupt PPI performance. The D_2 receptor family has been determined to be the more influential on PPI performance than the D_1 receptor family. This was concluded after administration of D_1 agonists or antagonists such as SCH23390 had little effect on sensorimotor gating performance in rats (Ralph et al., 2001).

Drugs that act as potent dopamine D_2 agonists such as apomorphine produce reductions in PPI, and dopamine antagonists such as haloperiodol have been shown to eliminate PPI deficits produced by apomorphine (Mansbach, Braff, & Geyer, 1989). Also, atypical antipsychotics-such as clozapine, olanzapine, quetiapine, and risperiodone-also reduced the impairments of PPI testing (Sipes & Geyer, 1997; Swerdlow et al., 2001; Varty, Baksi, & Geyer, 1999; Zhang, Bast, Feldon, & White, 2000). It is believed these typical and atypical antipsychotics are alleviating deficits because of their high affinity for the D_2 receptor (Geyer et al., 2001). Mice genetically engineered to lack the DA transporter, the DA protein that aids in the reuptake of DA into the presynaptic neuron, have significantly more DA in the synaptic cleft and, therefore, also show deficits in PPI performance (Serdlow et al., 2001).

Functionally, the D_2 receptor can be located presynaptically acting as an autoreceptor regulating release of dopamine. Postsynaptically, the D_2 receptor can exert a variety of functions, ranging from inhibiting of long-term depression at midbrain excitatory synapses, inhibiting of calcium channels, and controlling pacemaker activity and resting potential through activation of GIRK channels. These channels are G-protein-coupled inward rectifier potassium channels, which are activated by G-proteins (Hopf, Cascini, Gordon, Diamond, & Bonci, 2003). Physiologically, the D_2 receptor is located in many areas of the central nervous system but is

primarily located in the substantia nigra, ventral tegmental, and striatum, which include the nucleus accumbens shell and core and the dorsal striatum, olfactory tubercule, and the pituitary gland (Missale, Nash, Robinson, Jaber, & Caron, 1998).

Interestingly, humans with schizophrenia have been shown to have changes in D_2 receptor activity in the brain (Zimmerberg & Weston, 2002). It has been shown that dopaminergic hypoactivity in the frontal cortex and dopaminergic hyperactivity in the subcortical regions are major contributing factors to both positive and negative symptoms of schizophrenia (Haber & Fudge, 1997 as cited in Tizabi, Copeland, Brus, & Kostrzewa, 1999). Schizophrenia is associated with enhanced dopamine receptor sensitivity (Seeman et al., 2005). Also, antagonism of the D_2 plays a role in antipsychotic action. It is now well known that every antipsychotic drug must block D_2 receptors with some affinity to be clinically effective for schizophrenia (Tollefson, 1996).

Involvement of G-protein in Dopamine Receptor Signaling. The G-protein is a fundamental part in how the dopamine receptor works. A G-protein –linked receptor is named a G-protein because ligand binding causes a change in receptor conformation that activates a particular G-protein. The activated G-protein then binds to a target protein such as an enzyme or a channel protein that changes the target's activity. Exactly how the G-protein is activated is also a precise procedure that is essential to how the dopamine system functions. The major components of activation of a G -protein receptor is the conversion of GTP to GDP that determines the on or off state of the protein respectively. The G-protein can be separated into two classes: the large heterotrimeric G-proteins and the small monomeric G-proteins. The large heterotrimic G proteins contain three different subunits, G alpha (Gα), G beta (Gβ), and G gamma (Gγ).

RGS Proteins. The G-protein is first activated when the ligand binds to a metabotropic receptor that activates a G-protein by causing the Gα subunit to release GDP and obtain GTP. The Gα and Gβγ subunits then separate and initiate signal transduction events. Subsequently, the GTP-Gα subunit hydrolyzes its bound GTP, converting the subunit back to its inactive GDP-Gα form. This entire process is regulated by regulators of G-protein signaling, referred to by the acronym (RGS). These RGS proteins negatively regulate G-protein signaling by accelerating the rate of GTP hydrolysis catalyzed by G-proteins. There are at least 20 well-characterized RGS proteins in humans (Traynor and Neubig, 2005), and more than 30 mammalian RGS proteins that have been identified (Shelat et al., 2006). In the present study, we analyzed three different RGS proteins, RGS 4, 9, and 17, based on their involvement in dopaminergic receptor signaling and overall functioning of metabtropic receptors in the brain. Further, there has been very little information regarding the involvement of RGS proteins in behavioral tests known to be highly related to the dopamine system, such as prepulse inhibition. Finally, analysis of RGS proteins will provide important information as to whether the G-protein may be coupling to the dopamine D_2 receptor more efficiently in rats neonatally treated with quinpirole. To this point, the only method in which D_2 receptor priming has been determined in our laboratory has been through the yawning behavioral test.

Depending on their cellular localization and their specific interaction with the different Gα protein subunits or intracellular effectors, the RGS protein may specifically regulate certain receptor-mediated signaling cascades (Taymans et al., 2004). Relevant to this project, some G-protein signaling receptors, such as dopamine receptors, may themselves regulate the expression of certain RGS proteins. Taymans et al. (2004) have shown that specific dopamine receptor agonists and antagonists can regulate RGS2 or RGS4 mRNA in the rat striatum. For example, an

up-regulation of RGS2 has been reported when a D_1 agonist or D_2 antagonist was administered, whereas a D_1 antagonist or a D_2 agonist causes a down regulation of RGS2 and concurrent up-regulation of RGS4. Furthermore, they concluded that RGS2 and RGS4 have a large role in the enhancement of D_1 and D_2 receptor signaling cascades. Specifically, drugs that are D_2 agonists, such as quinpirole, act on presynaptic D_2 receptors to regulate RGS2 proteins and on postsynaptic D_2 receptors to regulate RGS4 proteins (2004). Therefore, it can be concluded that RGS4 has an influence on the mechanism of the G-protein receptor coupling at dopamine receptors and can be altered by dopaminergic agents.

Stanwood, Parlan, and Levitt, (2006) have suggested that RGS4 and RGS9 have an important role in the functioning of the dopamine system. After prenatal cocaine exposure, persistent increases in RGS9 were observed in the frontal cortex of the same mice when analyzed as adults. Specifically, RGS9 was found in higher densities in the striatal regions of brain, which include dorsal striatum, ventral striatum, and olfactory tubercle. The high levels of dopamine receptors expressed in these brain areas appear to indicate that RGS9 has an active role in the dopamine system (Rahman et al., 2003). Cabrera-Vera et al. (2004) describe RGS9 as a specific regulator of dopamine receptor-mediated signaling. Results from this study have shown that RGS9 regulates the dopamine receptor by reducing D_2 dopamine receptor modulation of calcium channels. Rahman et al. (2003) also concluded that an over expression of RGS9 decreases sensitivity to the behavioral effects of dopamine agonists, whereas a decrease of RGS9 significantly increases sensitivity to the behavioral effects of dopamine agonists.

Finally, RGS17 is a member of the RZ family, which is strongly expressed in cerebellum and other brain regions. All RZ family members reduce dopamine- D_2/G_i-mediated inhibition of cyclic adenosine monophosphate (cAMP) formation and abolish thyrotropin-releasing hormone

receptor/G_q-mediated calcium mobilization (Mao et al., 2004). RGS17 is a new RZ member that preferentially inhibits receptor signaling via $G_{i/o}$, G_z, and G_q over G_s to enhance cAMP-dependent signaling and inhibit calcium signaling. However, all research that has been performed thus far analyzing RGS 17 has been done in vitro with tranfected cells, and there is not any data as to whether RGS 17 is co-localized with dopamine D_2 receptors *in vivo*. Therefore, RGS17 is believed to have a general role in the overall coupling of the G-protein through the regulation of cAMP formation.

Therefore, RGS transcripts, specifically RGS4, 9, and17, have been shown to have a role in G-protein and dopamine transmission through regulation of the G-protein receptor in specific areas of the brain. Researchers are now examining certain psychopathologies, such as schizophrenia, in which G-protein receptors are also activated because of irregular dopaminergic receptor signaling (Erdely, Tamminga, Robers, & Vogel, 2006). For example, findings have shown a significant increase in RGS4 in the central nervous system of schizophrenics (Erdely et al., 2006; Morris et al., 2004; Prasad et al., 2005; Williams et al., 2004). As previously mentioned, RGS proteins have an active role in the dopamine system and analysis of these proteins highly related to the dopaminegic system should provide information as a possible mechanism of G-protein coupling in rats neonatally treated with quinpirole.

Nicotine

Nicotine also has a measurable effect on cognition and sensorimotor gating in humans with schizophrenia (Faraday, Rahman, Scheufele, & Grunberg, 1998). Heishman (1994) has reported that chronic administration of nicotine enhanced attentional processes in humans as well as in rats with cognitive deficits. Humans with schizophrenia are known to exhibit cognitive impairment (Elvevag & Goldberg, 2000). Various studies have shown that approximately 80% of

patients with schizophrenia smoke cigarettes as compared to 20% of the general population. Leonard et al.(1998) reported that 25% of the smokers in the United States were mentally ill. It has also been observed that people with schizophrenia appear to extract more nicotine from each cigarette than normal smokers, possibly because of different inhalation patterns (Olincy, Young, & Freedman, 1997). Researchers hypothesize that humans with schizophrenia are smoking as a form of self-medication for cognitive deficits (Kumari & Postma, 2005).

Some researchers believe that an increase in smoking may be because of certain antipsychotic medication (McEvoy,, Freudenreich, Levin, & Rose, 1995). Smoking has been shown to increase the metabolism of the typical antipsychotic haloperidol; whereas, the atypical antipsychotic clozapine appears to decrease the craving to smoke (1995). A hypothesis to explain this difference makes the proposition that medications interact in different ways with the nicotinic receptor in the brain. Although there is not a clear consensus concerning the interaction of antipsychotics with nicotine, there are numerous studies of how nicotine interacts, during sensorimotor gating testing in people with schizophrenia that are non-medicated. In humans nicotine has been found to alleviate sensorimotor gating deficits via the α7 nicotine receptor subtype. This receptor is believed to be playing a role in filtering auditory stimuli, which plays a major role in sensorimotor gating as tested by PPI.

Nicotine Receptor. Nicotinic receptors, or nicotinic acetylcholine receptors (nAChRs), are ionotropic receptors that open ion channels in the cells' plasma membrane. Similar to other types of acetylcholine (Ach) receptors, their opening is triggered by the neurotransmitter acetylcholine, but they are also opened by the Ach agonist nicotine (Siegel et al., 1999). There are many different subunits of the nicotinic receptor. These subunits belong to a multigene family (16 members in humans), which make different combinations to form different nAChRs. These

receptors with highly variable kinetic, electrophysiological, and pharmacological properties respond differently to nicotine at different concentrations. Nicotinic receptors can also be found post-synaptically, such as the muscular nicotinic receptor that always functions post-synaptically. However, in the brain, the receptor can be found both post-synaptically (involved in classical neurotransmission) and pre-synaptically, where they can influence the release of other neurotransmitters (Giniatullin, Nistri, & Yakel, 2005). Functionally, nAChRs in the CNS are considered to be of a more modulatory influence on general neurotransmission.

Activation of nicotinic receptors produces an increase of several neurotransmitters including dopamine (Wonnacott, 1997). Although nicotinic receptors can be located both pre- and post-synaptically, it is assumed that they aid in the release of dopamine pre-synaptically by increasing calcium influx into the cell. Calcium enters the cell through the calcium channel, which is opened by activation of the nicotinic receptor. This influx of calcium causes the neurosecretory vesicles to move closer to the plasma membrane. This is where the vesicles will fuse to the plasma membrane and then be released into the synaptic cleft (Berg & Conroy, 2002). Furthermore, nicotinic receptor interactions with dopaminergic neurotransmission in mesolimic and nigrostriatal pathways have been suggested to be responsible for locomotor sensitization to nicotine (Clarke, 1990; Richardson & Tizabi, 1994). Nicotine is believed to alleviate deficits in cognitive functioning in people with schizophrenia because nicotinic receptors in the hippocampus may be involved in cognitive functions such as attentional processes (Freedman et al., 1997) and working memory function (Elvevag & Goldberg, 2000). However, as noted earlier, nicotine is a mediator of downstream neurotransmission including an influx of dopamine that may play a role in cognition.

The nicotinic receptor is believed to have many different subunits; however, only a few major subtypes on nAChRs have been identified. These include the α4β2 nAChR, which is relatively abundant in the CNS and constitutes over 90% binding of high affinity [^{3}H]-cytisine binding in the rat brain (Barik & Wonnacott, 2006). The other major subtype of neuronal nAChR comprises α7 subunits, which are generally thought to form homomeric nAChRs in the CNS and peripheral nervous system. Therefore, the α7 receptor is one of the two most abundant nicotinic receptors found in the brain. Furthermore, a high number of synaptic α7- nAChRs have been found in the rat hippocampus, which is important in memory formation and relies on glutamate for excitatory signals (Berg & Conroy, 2002).

Researchers have shown that people with schizophrenia have a low number of α7 nicotinic receptors. That could hypothetically be a partial explanation of the deficits in sensorimotor gating (O'Neill, Rieger, Kem, & Stevens, 2003). Specifically, the ability to inhibit the response to the second auditory stimulus has been correlated with the number of α7 nicotinic receptors in the hippocampus in mice (2003). In receptor autoradiographic studies, a low number of α7 nicotinic receptors were found in the postmortem brain of humans with schizophrenia. This low affinity was an approximate 50% decrease as compared to normal brain tissue (Leonard et al., 2000). The specific areas of the brain with these low affinities were the hippocampus, cortex, striatum, and thalamus, which are all important in sensorimotor gating and other schizophrenic deficits in behavior. However, these results have been consistently varied depending on tested dose ranges or particular expression of the receptors in different strains of species and strains (Schreiber et al., 2002). What has been concluded is that the α7 receptors do have an important role in the effect of nicotine in sensorimotor gating; on the other hand, studies have shown that

more than one type of nicotinic receptor is playing a role in the mechanism of sensory gating in conjunction with nicotine administration, such as the α4/β2 nicotinic receptor.

The main differences in α7 and α4/β2 subtypes are the different levels of affinity of nicotine that differently affects these receptors. Nicotine has a higher affinity for the α4/β2-receptor subtype, while nicotine has a lower affinity for the α7 receptor (Court et al., 1998). It has been hypothesized that low doses of nicotine disrupt and high doses enhance PPI performance in rats and mice (Schreiber, Dalmus, & De Vry, 2002). Nicotine's lower affinity for the α7 receptor would suggest that lower does of nicotine would be activating that receptor, which could cause PPI deficits in rats. High affinity binding sites, α4/β2, are located in the striatum, substantia nigra, and presubiculum, and α4/β2 subunits were found in high levels in all thalamic nuclei (thalamus) (Ryan & Loiacono, 2000). Furthermore, α7, which has a lower affinity for nicotine, is located in the cerebral cortex and the hippocampus but not in the thalamus. Thus, both receptors likely play a role in PPI but likely play different roles based on their location in the brain and also may play different roles based on the dose of nicotine that is administered.

Gender Differences in Schizophrenia

Women with schizophrenia demonstrate a later age onset and higher premorbid and overall functioning (Goldstein, 1988; McGlashan & Bardestein, 1990; Symanski & Hertz-Picciotto, 1995). Women more often express affective symptomology and are more vulnerable to paranoia and hallucinations (Andia et al., 1995). Men more frequently exhibit flat affect and suffer from other negative or deficit symptoms (Symanski & Hertz-Picciotto, 1995). Although there is ample research that demonstrates gender differences in schizophrenia, there is very little information concerning whether there are gender differences in the impact of substance abuse in the schizophrenic population. Results of a recent study suggests that the higher overall functioning

observed in women with schizophrenia disappears with substance abuse (Gearon & Bellack, 2000), and there does not appear to be a difference in functioning between substance abusing women and men with schizophrenia. Research has further suggested that women with schizophrenia may be particularly vulnerable to the negative effects of substance abuse, a finding consistent with substance abuse in the general population (Le Duc & Mittleman, 1995).

Gender differences are also observed in behavioral testing such as PPI. Many past studies using PPI have only used males because female testing results have been observed to fluctuate with menstral cycles. Females demonstrate a deficit in PPI compared to males, with lowest level of PPI scores, during the midluteal phase of menstrual cycle (Swerdlow, Hartman, & Auerbach, 1997). In healthy participants, women have lower PPI scores than men (Kumari & Postma, 2005), therefore, resulting in lower scores. The same is found in people with schizophrenia, with women with schizophrenia having lower scores on average (Kumari & Postma). However, women with schizophrenia still vary in their scores throughout the month, again possibly because of the influx of estrogen and other hormones that could offer some protection against these sensorimotor gating deficits. Women with schizophrenia still exhibit significantly less PPI than women without schizophrenia (Swerdlow, 1997).

Statement of Problem

There are several unanswered questions in the current literature on the interaction of nicotine and PPI. First, in most PPI studies nicotine has been analyzed after acute administration, and there has been very little information on the effects of chronic nicotine on PPI. Second, most studies do not behaviorally test PPI over several days but test PPI in one day of testing (Swerdlow et al., 2001). The problem with these past studies is that PPI performance does significantly change over days in control animals, at least suggesting there may be learning of the

association between the prepulse, and the startle stimulus as learning is defined as a change in behavior due to experience. If there are significant changes, a key question is whether nicotine will affect PPI in later days of testing as compared to earlier days of testing and how that may compare within a rodent model of schizophrenia. This project is designed to analyze the effects of nicotine on PPI in a rodent model of schizophrenia.

The first question is: Does the nicotinic receptor mediate the effects of nicotine on PPI in D_2-primed male and female rats? From the information collected in the first experiment, it is known that nicotine has an affect on PPI performance; however, nicotine affects many different neurotransmitters through different receptors. Therefore, by using a specific nicotinic antagonist, mecamylamine, it will be possible to test the hypothesis that PPI performance at the nicotinic receptor by blocking the behavioral deficits caused by nicotine in PPI testing.
Furthermore, this study is designed to further understand the role of the D_2 receptor in the D_2 primed rodent model of schizophrenia by analyzing the RGS genetic transcripts in areas of the brain specific to dopamine functioning.

The second question is: What are the changes in genetic transcripts that code for the G-protein that couples to the D_2 receptor related to neonatal and adulthood drug treatments? Previous literature has stated that neonatal drug treatments of quinpirole permanently change the dopamine system, specifically the D_2 receptor (Brown et al., 2004). The D_2 receptor is a G-protein linked receptor, which is mediated by G-protein signaling protein RGS, which are proteins that negatively regulate G-protein signaling. RGS proteins accelerate the rate of GTP hydrolysis catalyzed by heterotrimic G-proteins (Traynor & Neubig, 2005). Specifically, studies have shown a significant increase in RGS4 in the central nervous system in schizophrenia (Erdely et al., 2006; Morris et al., 2004; Prasad et al., 2005; Williams et al., 2004) The RGS 7

transcript will be further significantly increased by adulthood nicotine treatment. Therefore by analyzing RGS transcripts, there will be more experimental support that quinpirole causes genetic changes in the dopamine system.

Hypotheses

Hypothesis 1. Neonatal quinpirole treatment will result in a reduction in PPI performance.

Hypothesis 2.Adulthood nicotine treatment will exacerbate PPI reduction in rats neonatally treated with quinpirole based on the fact that nicotine enhances dopamine function, increases in dopamine function has been shown to reduce PPI performance.

Hypothesis 3. We predict that sex differences will exist in PPI, with male rats demonstrating increased PPI performance as compared to female rats regardless of neonatal drug treatment. This prediction is based on past studies that have generally shown an improvement in PPI of male rats administered nicotine as compared to females (Acri,1994a; Acri, Brown, Saah, & Grunberg, 1995; Acri, Grunberg, & Morse, 1991; Acri, Morse, Popke, & Grunberg, 1994b)

Hypothesis 4.The nicotinic antagonist mecamylamine will block the effects of nicotine on PPI in D_2 -primed and non D_2-primed rats.

Hypothesis 5.There will be an increase in expression of the RGS4 transcript in the striatum and frontal cortex of rats neonatally administered quinpirole. There will be a decrease in RGS9 because RGS 9 has an important role with the D2 receptor. There will be an increase in RGS17 because RGS17 has an inhibitory role in dopamine signaling.

CHAPTER 2

METHOD

Main Experiment

Subjects

Four males and six female Sprague-Dawley rats were purchased from Harlan, Inc. (Indianapolis, IN). When received at East Tennessee State University (ETSU), each female was housed separately in a plastic polycarbonate cage with a male for approximately 7 days then separated. The offspring of each mating pair, a total of six litters, were the subjects in this experiment. There were six subjects in each group in the experiment, with one animal from each letter assigned to each of four drug treatment conditions. Animals were kept in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited climate-controlled animal colony with a 12-hour on/off light/dark cycle, and all testing was preformed during the light cycle. The University Committee on Animal Care at ETSU approved all procedures for this study.

Materials

Drugs. For neonatal drug treatment and verification of D_2 receptor priming, quinpirole HCI (Sigma-Aldrich, St. Louis, MO) was used. For neonatal drug treatment, a 100 μg/kg dose was used. The dose in neonatal drug treatment has been shown to produce priming of the D_2 receptor, and the 100 μg/kg dose has been shown to be sufficient to produce a significant increase in yawning in D_2 -primed rats. Nicotine was used first in adulthood drug treatment, and a dose of 0.5 mg/kg free base was administered. This dose was chosen as the preponderance of the research literature has shown that 0.5 mg/kg free base dose of nicotine is sufficient to produce sensitization to nicotine (Le Foll, Diaz, & Sokoloff, 2003). Second, a nicotine antagonist,

mecamylamine (1.0 mg/kg), or saline was administered to animals before nicotine or saline treatment.

PPI Apparatus. The PPI testing was performed in two sound-attenuated chambers on temporary loan from Bowling Green State University, which were originally purchased from San Diego Instruments (San Diego Instruments, San Diego, CA). The rats were placed in a cylindrical Plexiglas cylinder that were 10 cm in diameter and mounted on a platform inside this chamber located 25 cm below a high-frequency loudspeaker. A 70 dB white noise provided the background auditory stimulus. The animal was placed within the cylinder for testing. The animal was not restrained in these cylinders, and the animal was able to move and turn around within these cylinders. The startle response of the animal was measured through a unit mounted underneath the Plexiglas cylinder that sent an analog piezoelectric signal to the computer that was digitized and stored on the computer. Calibrations were performed before behavioral testing to maintain accurate acoustic stimuli presentation and mechanical-vibration measures.

Design and Procedure

For analysis of yawning behavior, a 2 x 2 ANOVA was used, with sex (between subjects variable: male, female), and neonatal drug treatment (between subject variable: quinpirole, saline) as the two factors. For both the mean startle response and PPI, performance was analyzed on days 1, 4, 7, and 10, identical to the data analyses used by Culm et al. (2004a; 2004b) that also used a 10-day PPI testing procedure. The mean startle response was analyzed using a 2 x 2 x 2 x 4 ANOVA with sex (between subjects variable: male, female), neonatal drug treatment (Between subjects variable: quinpirole, saline), adulthood drug treatment (between subjects variable: nicotine, saline), and day of testing (within subject variable: day of testing, four levels) as the four factors. The PPI collective data of mean startle response was initially analyzed using

a 2 x 2 x 2 x 3 x 4 ANOVA with sex (between subjects variable: male, female), neonatal drug treatment (between subjects variable: quinpirole, saline), adulthood drug treatment (Between subjects variable: nicotine, saline), prepulse auditory intensity (within subjects variable: 73 dB, 76 dB, 82 dB prepulse) and day of testing (within subject variable: day of testing, four levels). If there were any significant effects involving gender, then males and females were analyzed separately. Any significant interactions involving auditory intensity of the prepulse resulted in separate ANOVAs on each prepulse auditory intensity. Fisher's Least Significant Difference test was used for any post-hoc comparisons.

Neonatal Drug Treatment. The day of birth was counted as post-natal day 0 (P0). Beginning on P1, the rats received once daily i.p. injections of either quinpirole (1mg/kg) or saline for the next 21 consecutive days (see Table 1). All animals were weaned from the female dam at P21 and socially housed, two to three per cage. Food and water was available ad libitum.

Yawning Procedure. Yawning is a measurement of D_2 receptor sensitivity, and increased yawning is considered a manifestation of dopamine D_2 receptor activation as it is a D_2 receptor mediated behavioral event (Cooper, Rusk, & Barber, 1989). Therefore, dopamine D_2 receptor supersensitization was verified through a single i.p. injection of quinpirole (100mg/kg) at approximately P65, and yawning behavior was observed for 1 hour. Two observers recorded yawning and were blind to the group the animals were assigned. During the yawning test, animals were placed in a cage without bedding because animals tend to gnaw on the bedding, and this behavior interferes with yawning. The number of yawns were counted for each animal for 1-hour period (see Table 1).

Adulthood Drug Treatment. Those animals assigned to the nicotine treatment group began nicotine treatment before PPI to become sensitized to the drug. This was done for 5 days to

alleviate the hypoactive initial behavioral response that is the characteristic response to initial administrations of nicotine (Dwoskin, Crooks, Teng, Green, & Bardo, 1999). Adulthood nicotine treatment before PPI testing began from P66-P70. On each day of PPI behavioral testing, each animal received either an injection of nicotine (0.5 mg/kg free base) or saline 15 minutes before PPI testing.

Mecamylamine (1.0 mg/kg) was administered to the animal during the 5-day nicotine sensitization period. During behavioral testing, mecamylamine or saline was administered 15 minutes before nicotine or saline treatment. Fifteen minutes later, the animal was placed into the PPI chambers and tested (see Table 1).

Table 1

Complete Drug Treatment Regimen and Research Design.

	Neonatal Drug Treatment	Yawning Behavioral Test	Adulthood drug treatment pre-PPI Testing		Adulthood drug treatment during PPI testing	
Drug Group	P 1-21	P 65	P 66-70		P 71-80	
Q-SS	Quin 1mg/kg	Quin 100 μg/kg	Saline	Saline	Saline	Saline
Q-SN	Quin 1mg/kg	Quin 100 μg/kg	Saline	Nicotine 0.5 mg/kg	Saline	Nicotine 0.5 mg/kg
Q-MN	Quin 1mg/kg	Quin 100 μg/kg	Mec 1mg/kg	Nicotine 0.5 mg/kg	Mec 1mg/kg	Nicotine 0.5 mg/kg
S-SS	Saline	Quin 100 μg/kg	Saline	Saline	Saline	Saline
S-SN	Saline	Quin 100 μg/kg	Saline	Nicotine 0.5 mg/kg	Saline	Nicotine 0.5 mg/kg
S-MN	Saline	Quin 100 μg/kg	Mec 1mg/kg	Nicotine 0.5 mg/kg	Mec 1mg/kg	Nicotine 0.5 mg/kg

Abbreviations: Quinpirole (Quin); Mecamylamine (Mec); Postnatal day(P). Note: Brain tissue from Groups Q-SS and S-SS were harvested for analysis of RGS transcripts at P81.

PPI Procedure. PPI testing began 6 days later after verification of D_2 supersensitization via the yawning behavioral test. We used a SR-LAB startle reflex system (San Diego Instruments, San Diego CA) to measure the startle response. At the beginning of each trial, all animals were placed into the cylindrical animal enclosure and then were exposed to a 70 dB white noise for a five-minute acclimation period. The acclimation period was then immediately followed by a test session consisting of the randomized presentation of 35 trials. Of these 35 trials, the first 5 trials of each daily session were pulse trials, which were used to habituate the animal to the pulse. This was also done to eliminate large individual differences that exist because of initial presentation of the pulse trials (unpublished observations). After the presentation of the five pulse trials, 30 trials were presented in which 15 were pulse trials and 15 were prepulse trials. A pulse trial consisted of a high intensity startle auditory stimulus that was 115 dB in auditory intensity and persisted for 40-ms. There were 15 prepulse trials that consisted of a prepulse auditory stimulus of 73, 76, or 82 dB intensity that was 20-ms in length given 100-ms before the 40-ms 115 dB pulse. The mean response after each pulse and prepulse trial was then recorded for 100 ms after the stimulus was administered.

RGS analysis. After behavioral testing was complete, brain tissue was harvested and snap frozen in cold isopentane and stored in a -80^{o}C freezer in our laboratory (So Low, Cincinnati, OH). Brain tissue was sent to Vanderbilt University and RGS transcripts were analyzed using in situ hybridization technique. RGS transcripts 4, 9, and 17 in the striatum, frontal cortex, and nucleus accumbens of rats only neonatally treated with quinpirole or saline were examined. These three different RGS transcripts have been shown to be important in regulating the G-protein of both D_1 and D_2 dopamine receptors. Slides that were treated for in situ hydridization were sent back to our laboratory for densitometry analysis

CHAPTER 3

RESULTS

Prepulse Inhibition Overall Results

An initial 2 x 2 x 2 x 3 x 4 ANOVA was performed to analyze significant differences across all days of testing and all auditory intensities. This ANOVA revealed a significant main effect of neonatal drug treatment, $F(1,48) = 4.1$, $p < .04$, adulthood drug treatment $F(2,48) = 5.68$, $p < .006$, and auditory intensity, $F(11,48) = 28.03$, $p < .001$. Significant two-way (Adulthood Drug Treatment x Auditory Intensity) interactions $F(22,48) = 1.69$, $p < .02$ (Sex x Auditory Intensity) $F(11,34) = 1.80$, $p < .04$ and (Sex x Adulthood Drug Treatment), $F(2, 48) = 6.19$, $p < .004$. Based on the significant interactions with auditory intensity as well as sex, we decided to analyze the different auditory intensities separately for males and females, which are presented below.

73 dB Prepulse Trials: Males

The 73 dB prepulse results for males are presented in Figure 1. A 2 x 3 x 4 ANOVA revealed a significant main effects of neonatal drug treatment, $F(1,22) = 9.067$, $p < .006$, adulthood drug treatment, $F(2,22) = 6.583$, $p < .006$, and day of testing, $F(3,22) = 21.789$, $p < .0001$ as well as a significant two-way (Adulthood Drug Treatment x Day of Testing Interaction) interaction, $F(6,22) = 3.269$, $p < .007$,and a significant three-way (Neonatal Drug Treatment x Adulthood Drug Treatment x Day of Testing) interaction, $F(6,22) = 2.62$, $p < .02$. Neonatal quinpirole treatment produced a significant reduction in PPI at days 4, 7, and 10 and nicotine exacerbated reductions in PPI produced by neonatal quinpirole treatment at Day 10. Interestingly, the nicotinic receptor antagonist mecamylamine did not block the effects of nicotine at days 4 and 7 but did block the effects of nicotine at day 10. These results indicate that neonatal quinpirole treatment produces a significant reduction in PPI that is exacerbated by nicotine treatment. The

fact that mecamylamine a nicotinic antagonist, only partially blocked the effects of nicotine appears to indicate that the nicotinic receptor may not be completely mediating the effects of nicotine on PPI, and side effects of nicotine may be involved in these effects.

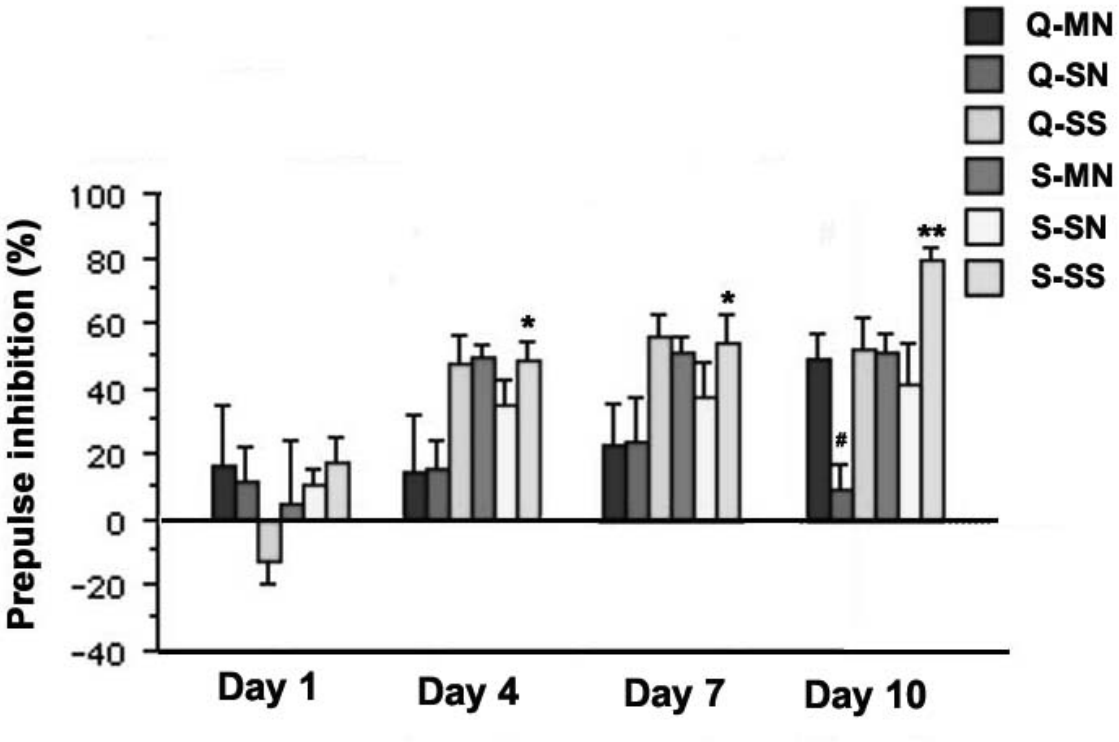

Figure 1. Percentage of PPI by Day of Testing for 73dB for Males

73 dB Prepulse Trials: Females

The 73 dB prepulse trial results for females are presented in Figure 2. In female rats, a 2 x 3 x 4 ANOVA revealed only a significant adulthood drug treatment main effect $F(2,26) = 3.73$, $p < .04$. Nicotine produced an overall significant increase in PPI as compared to controls.

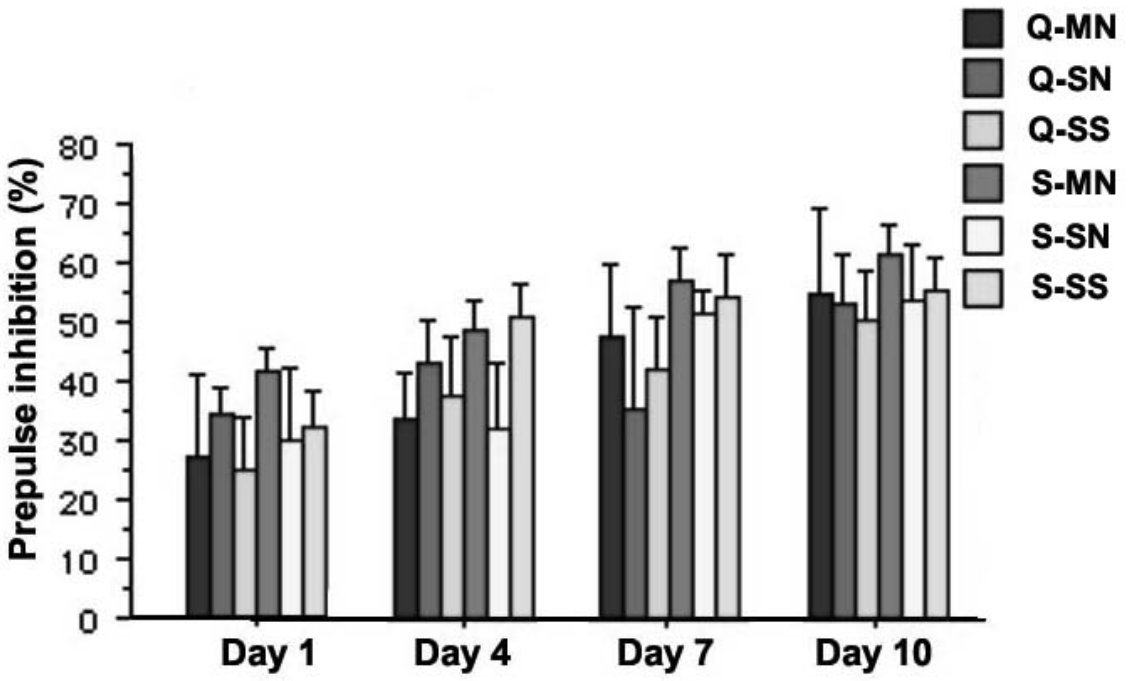

Figure 2. Percentage of PPI by Day of Testing for 73dB for Females

76 dB Prepulse Trials: Males

The 76dB prepulse trials for males are presented in Figure 3. A 2 x 3 x 4 ANOVA revealed significant main effects of adulthood drug treatment, $F(2,22) = 13.241$, $p < .0002$, and day of testing, $F(3,22) = 10.5$, $p < .0001$, and the neonatal main effect approached significance $p=.08$. In male rats, nicotine produced a significant deficit as compared to controls, and this effect was not alleviated by pretreatment with the nicotinic receptor antagonist mecamlyamine. Similar to the effects in 73 dB prepulse trials, mecamylamine did not completely alleviate the behavioral effects of nicotine, suggesting a side effect of this drug on PPI.

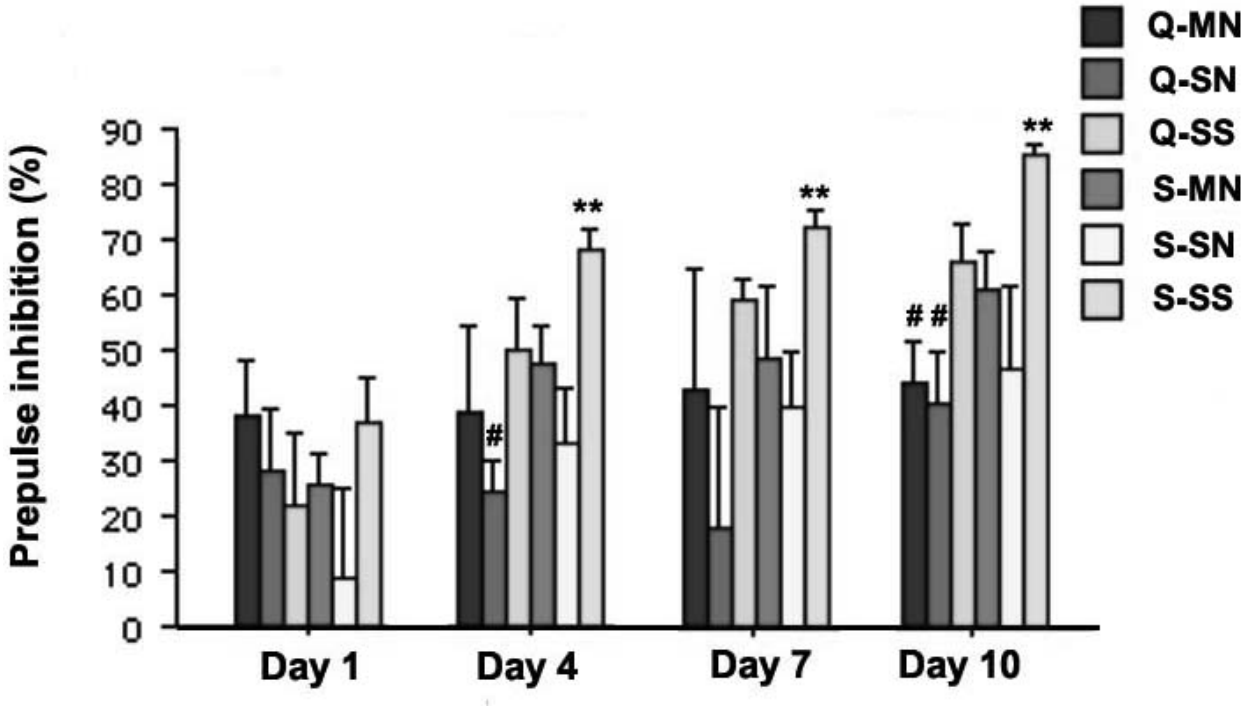

Figure 3. Percentage of PPI by Day of Testing for 76dB for Males

76 dB Preulse Trials: Females

The 76 dB prepulse trials for females are presented in Figure 4. No significant main effects or interactions were found for the 76dB prepulse trials in females. Unlike males, neonatal quinpirole treatment does not produce a significant reduction in PPI on 76 dB prepule trials in females, and nicotine also does not appear to affect PPI for this particular prepulse auditory intensity. This may in part be because of the relatively poor performance in female controls in PPI.

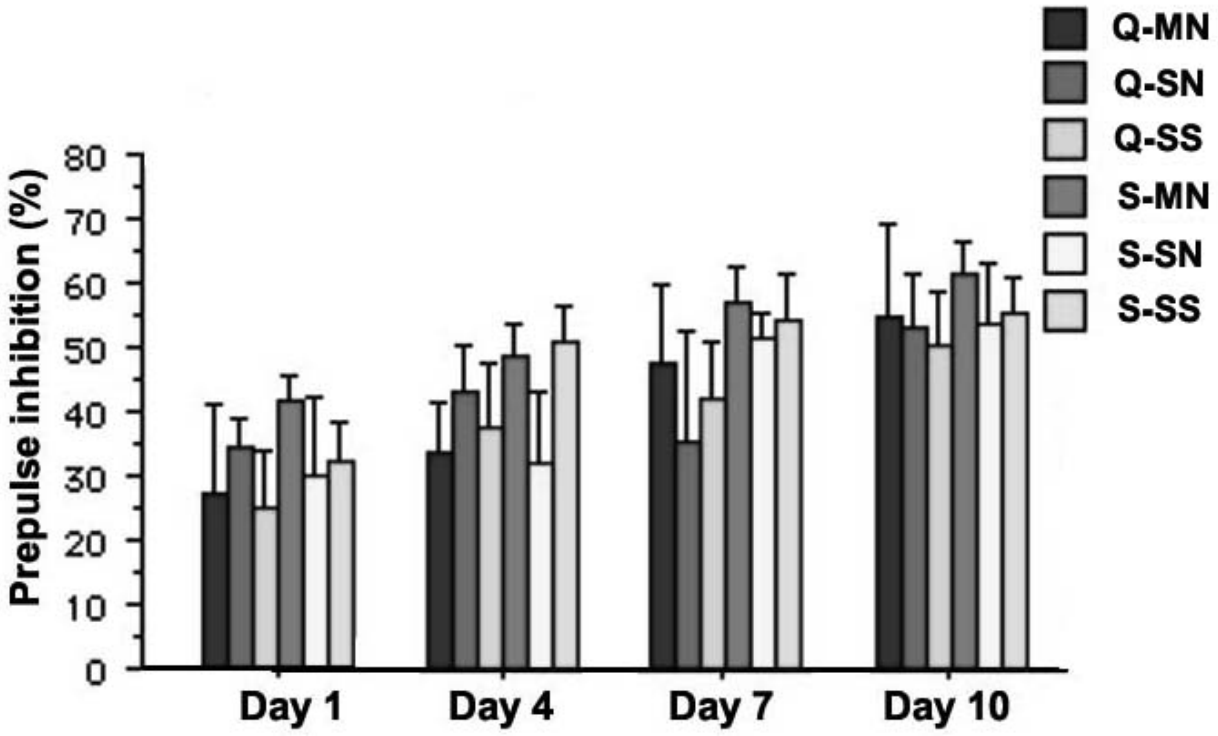

Figure 4. Percentage of PPI by Day of Testing for 76dB for Females

82 dB Prepulse Trials: Males

The 82 dB prepulse trials for males are presented in figure 5. A 2 x 3 x 4 ANOVA revealed significant main effects of adulthood drug treatment, $F(2,22) = 6.036$, $p < .008$, and day of testing, $F(3,22) = 13.224$, $p < .0001$, as well as significant two-way (Neonatal Drug Treatment x Day of Testing) interaction, $F(3,22) = 2.955$, $p < .038$, and (Adulthood Drug Treatment x Day of Testing) $F(6,22) = 2.852$, $p < .0157$. In male rats, neonatal quinpirole produced a significant reduction in PPI at days 1, 7, and 10. Nicotine exacerbated reductions in PPI produced by neonatal quinpirole, at days 7 and 10, and in contrast to the 73 and 76 dB prepulse trials, mecamylamine blocked the effects of nicotine across all days of testing.

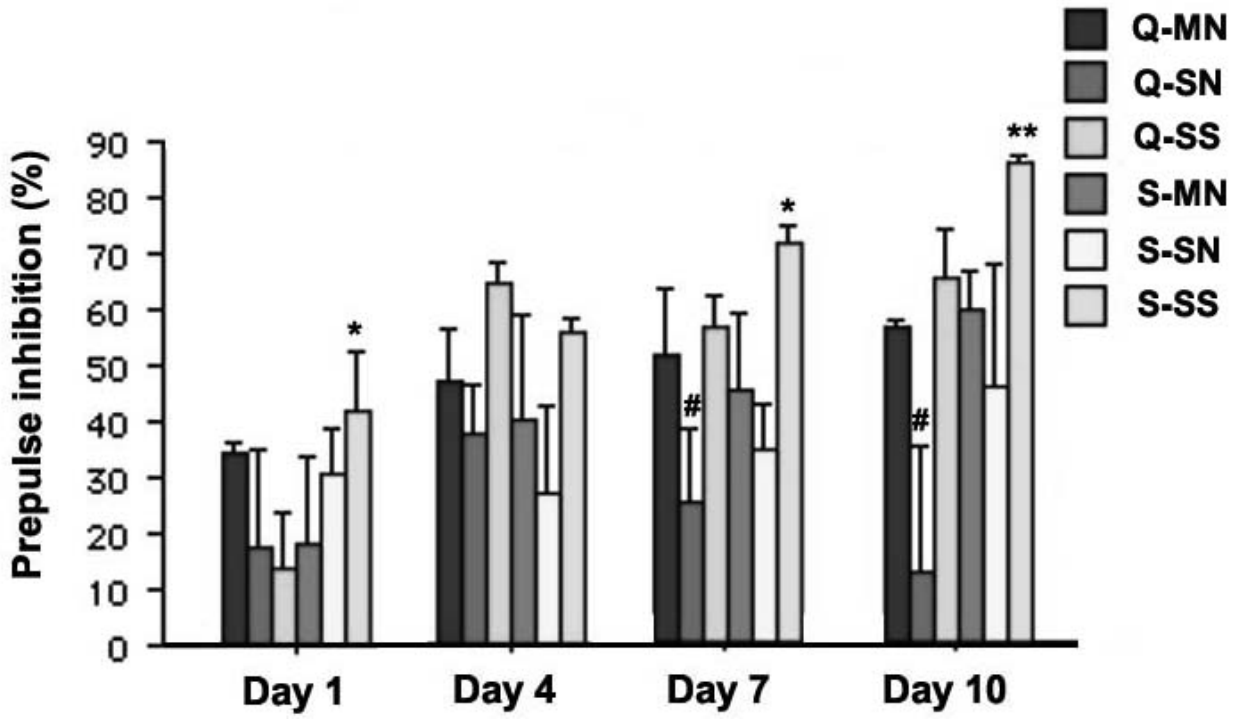

Figure 5. Percentage of PPI by Day of Testing for 82dB for Males

82 dB Prepulse Trials: Female

The 82 dB prepulse trials are presented in Figure 6. No significant main effects or interactions were revealed for 82 dB prepulse trials in females. Similar to the results of the 76 dB prepulse trials, neonatal quinpirole treatment does not produce a significant reduction in PPI on 82 dB prepule trials in females, and nicotine also does not appear to affect PPI for this particular prepulse auditory intensity.

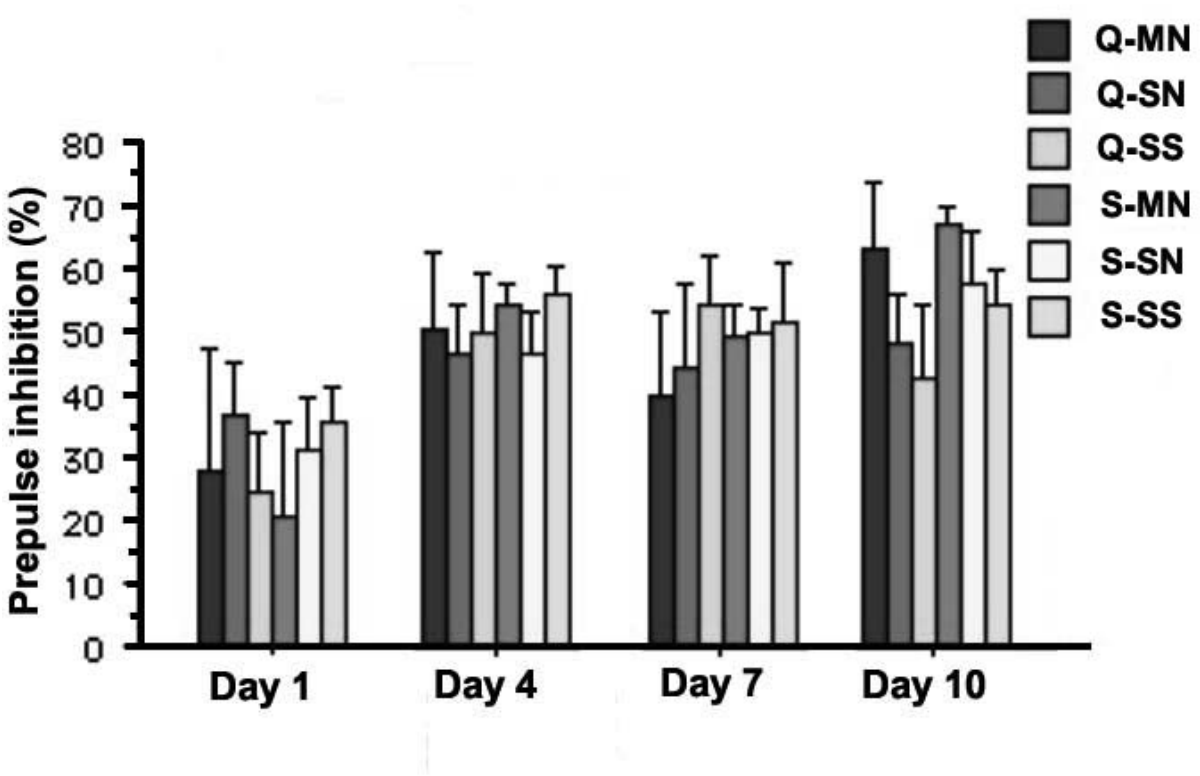

Figure 6. Percentage of PPI by Day of Testing for 82dB for Males

RGS Transcripts Results

RGS transcripts were analyzed only in animals neonatally treated with quinpirole and administered saline in adulthood (Group Q-SS) and controls neonatally treated with saline neonatally and in adulthood (Group S-SS). RGS4 was only analyzed in the frontal cortex and caudate nucleus and not nucleus accumbens because densitometry readings were weak for RGS4

in nucleus accumbens and they could not be analyzed. RGS transcript results are presented for RGS 4, 9, and 17 in Figures 7, 8, and 9. In situ hybridization revealed no significant change in microdensitometry readings for RGS4 in the frontal cortex, caudate nucleus. (see Figures 7a, b). There was no significant change in RGS9 due to neonatal drug treatment in the frontal cortex. However, neonatal quinpirole produced significant decreases in RGS9 as compared to controls in the caudate nucleus, $t(10) = 2.617$, $p < .028$ and the nucleus accumbens, $t(10) = 3.653$, $p < .015$ (see Figures 8a, b, c) These results are especially important to our model, as this demonstrates that the regulatory mechanism for the G-protein that couples to the D_2 receptor is reduced by neonatal quinpirole treatment, suggesting that the D_2 receptor is indeed hyperactive in these animals in two brain areas known to be heavily innervated with dopamine. Regarding RGS17, rats that were neonatally treated with quinpirole demonstrated a significant increase in RGS17 expression as compared to controls in the frontal cortex, $t(10) = 3.34$, $p < .01$, caudate nucleus, $t(10) = 2.94$, $p < .02$, and nucleus accumbens, $t(9) = 2.61$, $p < .04$ (see Figures 9a,b,c). Based on the fact that RGS17 has never been co-localized with the D_2 receptor, this may be a compensatory mechanism in other systems in these brain areas relative to neonatal drug treatment.

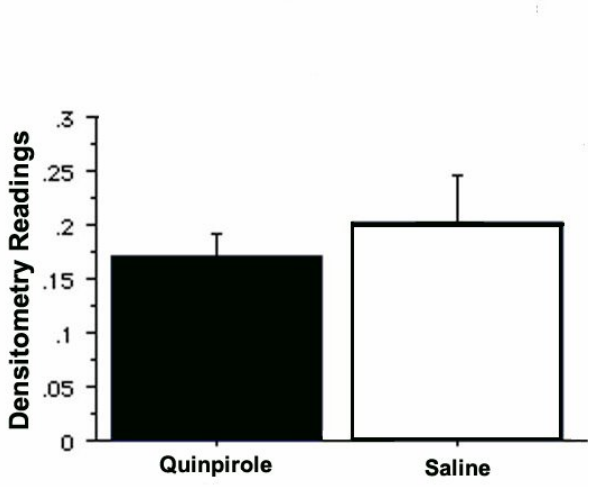

*Figure 7a.*RGS4 Densitometry Readings in Frontal Cortex

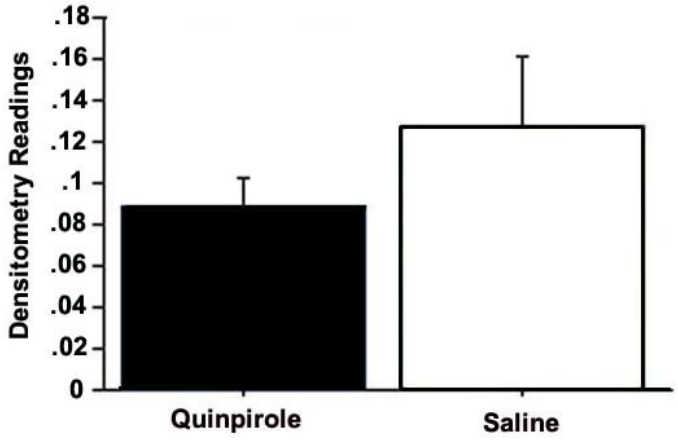

Figure 7b. RGS4 Densitometry Readings in Caudate Nucleus

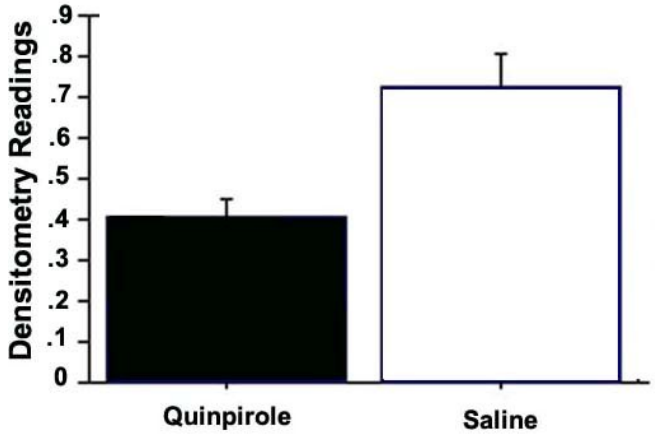

Figure 8a. RGS9 Densitometry Readings in Frontal Cortex

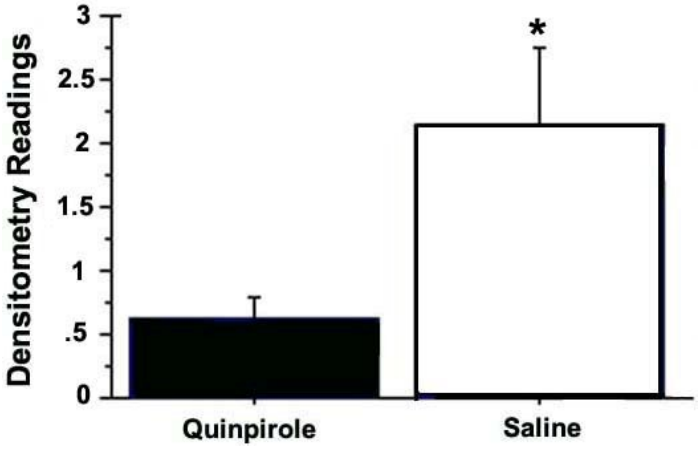

*Figure 8b.*RGS9 Densitometry Readings in Caudate Nucleus

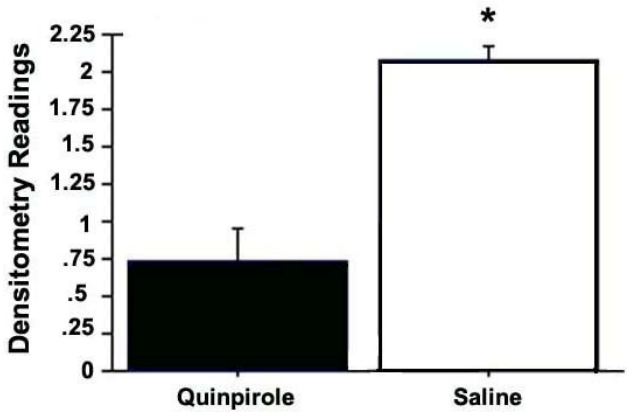

Figure 8c. RGS9 Densitometry Readings in Nucleus Accumbens

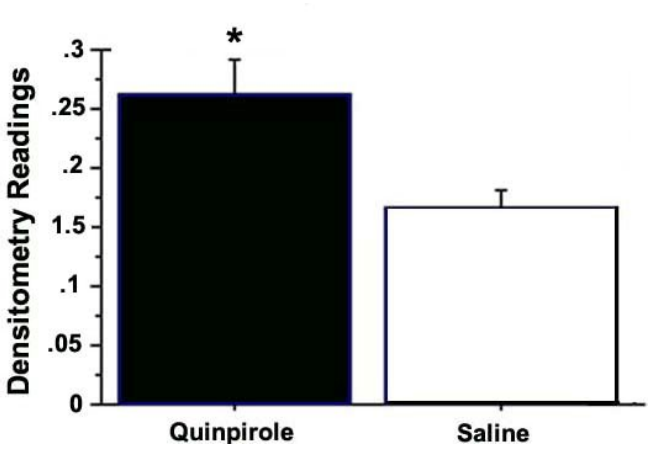

Figure 9a. RGS17 Densitometry Readings in Frontal Cortex

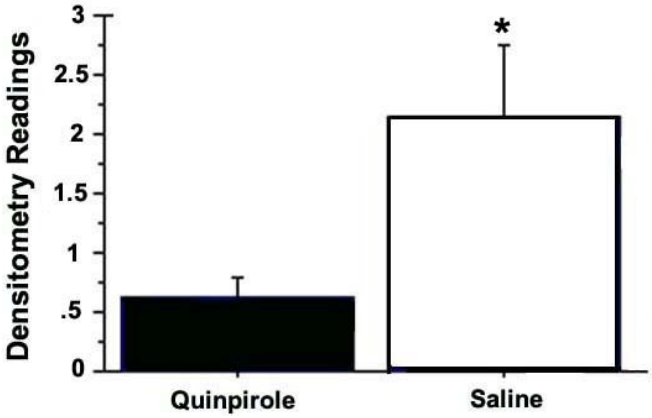

Figure 9b. RGS17 Densitometry Readings in Caudate Nucleus

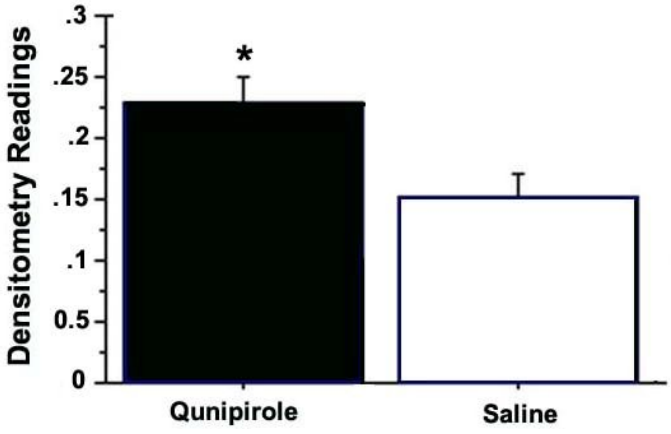

Figure 9c. RGS17 Densitometry Readings in Nucleus Accumbens

CHAPTER 4

DISCUSSION

In the current study, significant reductions in PPI functioning were observed in rats neonatally treated with quinpirole. Male rats neonatally treated with quinpirole demonstrated significant reductions in PPI compared with controls on 73, 76, 82 dB prepulse trials, whereas females did not show a significant reduction in PPI because of neonatal drug treatment. This result supports the hypothesis that neonatal quinpirole would produce a significant reduction in PPI functioning, therefore, replicating past findings from this laboratory (Smith, Thompson, Thacker, Perna, & Brown, 2004). Additionally, nicotine exacerbated reductions in PPI at different points across the different auditory intensity, suggesting that nicotine further stimulates an already hyperactive dopaminergic system to produce significant reductions in PPI. Interestingly, the nicotinic antagonist mecamlyamine only partially blocked the effects of nicotine, suggesting that nicotine's effects may be mediated by systems not mediated by the nicotinic receptor. Findings also revealed a significant sex difference in PPI, with males performing significantly better than females across all auditory intensities. This result supports past findings by Faraday et al. (1998) that have shown sex differences in PPI, but in this past study, animals were tested in 1 day. In the present study, animals were tested across 10 days of testing, but extended testing did not improve PPI performance in females. There was no significant difference between controls and experimental females possibly because of the overall poor performance of females.

The results are in agreement with past literature that have shown activation of the dopamine D_2 receptor results in significant reductions in PPI (Geyer et al., 2001; Swerdlow et al., 2001). Past studies from a collaborating laboratory have shown that neonatal quinpirole treatment

permanently hypersenzitises the D_2 pre and postsynaptic receptors in rats (Nowak et al., 2001, 2002). Increases in dopamine D_2 sensitivity occurs in several clinical conditions that have been shown to produce reduction in PPI, including schizophrenia and ADHD (Geyer et al., 2001). It is known that the D_2 receptor plays an important role in PPI as antipsychotic medications that block the D_2 receptor also alleviate these reductions in PPI. However, in the current study blockade of the D_2 receptor was not analyzed, so it cannot be determined whether the reduction in PPI is because of priming of the D_2, receptor because of neonatal quinpirole treatment, or some other side effects of this treatment. For example, past findings in this laboratory have shown that neonatal quinpirole treatment produces significant decreases of choline acetyltransferase (ChAT) and the neurotrophic factors nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the hippocampus of adult rats (Brown, et al., 2004). Reductions in cholinergic functioning have also been shown to reduce PPI performance (Jones & Shannon, 2000). Therefore, the effects reported here could be because of priming of the D_2 receptor or some other modulatory effect of neonatal drug treatment.

In the present study, nicotine actually exacerbated the reductions in PPI. This result is in contrast to past literature that has shown nicotine alleviates reductions in PPI in both humans and animals (Geyer et al., 2001; Leonard et al., 1998). However, a major methodological difference in past studies as compared to the current study was that subjects were only tested for 1 day. It is clear from the results presented here that there are changes in PPI performance across days of testing, and all groups increased PPI performance later in testing. Additionally, nicotine is known to produce significant increases in dopaminergic functioning, which, according to the literature should produce a *reduction* in PPI as drugs that increase dopaminergic function typically reduce PPI performance (Geyer et al., 2001, Swerdlow et al., 2000). It appears that as animals

continued to sensitize the effects of nicotine, as performance worsened across all three auditory intensities with further testing.

Hypothesized Mechanism of Nicotine in D_2-primed Rats

The hypothesized mechanism of nicotine on the dopaminergic system in D_2-primed rats is described in Figure 10. It can be hypothesized that the reduction in PPI produced by nicotine is caused by an increase of dopaminergic activity in the brain, (David & Abraini, 2001). It is well known that nicotine increases dopamine release by acting at the presynaptically located nicotinic receptors (nAChRs) (Brody et al., 2006). It has recently been discovered that nicotine also inhibits the functioning of the D_2 autoreceptor (Harsing, Sershen, & Lajtha, 1992), which is responsible for inhibiting dopamine release at the synapse. Essentially, via this mechanism, nicotine allows dopamine to stay in the synaptic cleft, and this mediates the positive reinforcing effects of nicotine. When nicotine binds to the presynaptic nicotinic receptor, calcium is allowed to enter the presynaptic terminal, binding to the protein calmodulin and carrying synaptic vesicles containing dopamine to the cell membrane.

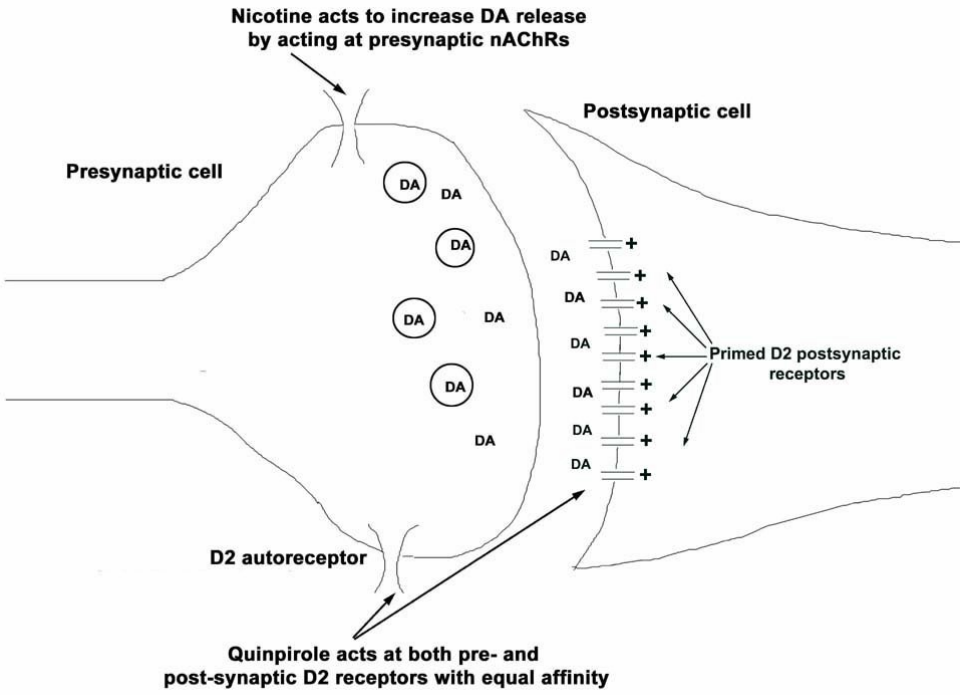

Figure 10. Synapse Diagram of Nicotine's Effect at D_2 Receptor

Dopamine is then released from these vesicles into the synapse, binding to primed dopamine D_2 receptors, increasing the overall dopaminergic response in D_2-primed rats given nicotine. The increase in overall dopaminergic response would presumably produce a significant reduction in PPI.

Previous literature has shown that nicotine can affect many different neurotransmitters in the brain, therefore, causing different behavioral effects (Giniatullin et al., 2005). Therefore, to confirm that nicotine action at the nicotinic receptor is mediating the effects on PPI, animals in the present study were pre-treated with the nicotinic antagonist mecamylamine to block the effects of nicotine on PPI. Mecamylamine only partially blocked the effects of nicotine, suggesting a side effect of nicotine may be mediating its effects on PPI, or that nicotine's effects on other neurotransmitter systems may be mediating these effects. On the other hand, it could be that the dose of mecamylamine was not high enough to completely block the effects of nicotine

throughout testing, although this dose has been used in the past to block nicotine locomotor sensitization (Miller, Wilkin, Bardo, Crooks, & Dwoskin, 2001). The fact that mecamylamine did not produce a reduction on PPI in controls is consistent with the past literature that has shown a similar effect (Jones & Shannon, 2000).

In the current study, RGS transcripts were analyzed using the in situ hybridization technique to investigate G-protein activity in dopamine D_2 receptors as well as general G-protein activity in the brain. Microdensitometry readings revealed a significant relationship between RGS9 and 17 levels and neonatal drug treatment. Rats neonatally treated with quinpirole demonstrated a significant decrease in RGS9 in the caudate and nucleus accumbens. RGS9 is described as a specific RGS for the regulation of the dopamine receptor because these proteins have been shown to be co-localized with dopamine D_2 receptors (Cabrera-Vera et al., 2004). As previously mentioned, Rahman (2003) concluded that an *increase* in RGS9 occurs when there is a *decrease* in sensitivity of dopaminergic receptors. In the current study, RGS9 was significantly *decreased* in these heavily innervated dopaminergic brain areas. Based on Rahman's findings (2003), this decrease in RGS9 suggests an increase in dopamine D_2 receptor activity. Past results have shown that neonatal quinpirole treatment produces a significant increase in D_2 sensitivity but does not produce an overall increase in D_2 receptor number (Kostrzewa et al., 1995). Therefore, this would appear to indicate that the G-protein might be coupling more efficiently with sensitized D_2 receptors in rats neonatally treated with quinpirole. A future study will analyze whether G-protein coupling is significantly increased using a more specific technique, the GTP-gamma-S assay, which is sensitive to increases in coupling of the G-protein.

The RGS17 protein was significantly increased across all brain areas analyzed as compared to controls: the frontal cortex, caudate nucleus, and nucleus accumbens in rats neonatally treated

with quinpirole. There has been less research performed on RGS17. What is known is that the RGS17 protein is important for the inhibition of the second messenger system cAMP. Findings have shown that RGS17 is a general regulator of the G-protein throughout the brain and has been shown to be associated with metabotropic receptors other than dopamine D_1 and D_2 receptors such as cholinergic muscarinic receptors (Mao et al., 2004). The significant increase in RGS17 observed in animals neonatally treated with quinpirole could be the result of a change at the D_2 receptor, but this change could be modulating other systems resulting in significant increases in the RGS17. For example, results from our laboratory have shown that neonatal quinpirole treatment results in significant decreases in the cholinergic system (Brown et al., 2004), and this result is consistent with those findings. However, there could be changes in other neurotransmitter systems that have not yet been analyzed that could also be producing this increase. Currently, no study has ever co-localized RGS17 with the D_2 receptor in native tissue, and this will also be the focus of a future study.

A main issue to keep in consideration in this study is that one neurotransmitter, such as dopamine, has not been the only neurotransmitter involved in PPI performance. As previously mentioned, other neurotransmitters, such as serotonin and norepinephrine, also have been shown to play a role in PPI and sensorimotor gating (Geyer et al., 2001; Swerdlow et al., 2001). Although quinpirole is a specific D_2/D_3 agonist, one cannot assume that other neurotransmitters and receptors are not being directly or indirectly influenced by priming of the D_2 receptor via neonatal quinpirole treatment. However, in the current study the main focus is the examination of dopamine's role in PPI functioning and the correlations that can be made to humans with schizophrenia, such as a supersensitized dopamine system (Einat et al., 1993). Although neonatal administration of quinpirole does not replicate all the brain abnormalities found in human with

schizophrenia, it can still be considered a valid model for information because it does model one aspect of the disease (Woodruff & Baisden, 1994). The current study supports findings that have shown neonatal quinpirole administration is a valid rodent model of schizophrenia via modeling replicating the supersensitized postsynaptic dopamine receptor and PPI deficits, which are both found in humans with schizophrenia.

In the current study, nicotine exacerbated PPI deficits in a rodent model of schizophrenia. This finding contradicts studies that have shown nicotine enhanced PPI in human patients with chronic schizophrenia (Kumari & Postma, 2005; Leornard et al., 2000). The findings in these studies along with the fact that 80% of humans with schizophrenia smoke cigarettes led many researchers to believe that humans with schizophrenia may be smoking to self medicate their auditory sensorimotor gating deficits (Leonard et al.). One reason for the contradiction between the current study and previous literature could be that past studies have used a 1 day behavioral testing methodology in PPI, and initial effects of nicotine contrast to the drug's effects after chronic administration (Geyer et al., 2001). Thus, these findings show that nicotine may be exacerbating these auditory sensorimotor gating deficits. Research has also suggested that schizophrenics may self-medicate because of cognitive deficits produced by the disorder (Elvevag & Goldberg, 2000). Cognitive deficits in schizophrenia have been suggested to be related to impairments in PPI (Geyer et al., 2001). However, the current study sought to examine the effects of nicotine on a D_2 primed rodent model of schizophrenia that exacerbated PPI deficits. In humans with schizophrenia, nicotine could be increasing dopamine, which could increase overall arousal, which may be causing them to feel better overall. Finally, it should also be considered that when humans with schizophrenia smoke cigarettes, they are inhaling many other chemicals in addition to nicotine that may be causing additional behavioral effects. The

current study gives valuable information into behavioral effects of nicotine given chronically in conjunction with a neonatal quinpirole induced rodent model of schizophrenia.

Future Studies

There are many different studies that can be generated from the findings of the current study. One of the more interesting findings of the study is nicotine's exacerbation of PPI deficits in a quinpirole-induced model of schizophrenia. A future study could focus on dopamine release in the dorsal caudate (also referred to as the striatum) using the microdialysis technique to analyze whether nicotine significantly increases dopamine in these animals. A second experiment could analyze whether mecamylamine, using the current dose of 1.0 mg/kg, blocks the effects of nicotine on dopamine release. If this were the case, then changes in other neurotransmitter systems may be implicated in nicotine effects on PPI. Another future experiment could analyze whether serotingergic, noradrenergic, or glutamatergic antagonists block the effects of nicotine on PPI more effectively than mecamylamine, which would implicate these other systems. Also, it may be useful to administer different dose ages of mecamylamine to determine an effective dose that would block nicotine's effect on PPI testing. It may also be interesting to investigate the specific nicotinic receptor that is being affected by nicotine. As previously mentioned, nicotine works on different nicotinic receptors, and findings have shown that different nicotine receptors may be play differential roles in PPI (Leonard et al., 2002). This could be done by using a specific nicotinic receptor antagonists that are specific to the $\alpha 7$ or $\alpha 4/\beta 2$ nicotinic receptors. In terms of the findings of RGS transcripts, it would also be interesting to examine GTP-gamma-S in these different brain areas to determine whether the G-protein is indeed coupling more efficiently to the D_2 receptor.

In conclusion, this study demonstrates a deleterious effect of nicotine utilizing a novel rodent model of schizophrenia. Although these findings are in contrast to the effects of nicotine on PPI in schizophrenia (Geyer et al., 2001; Swerdlow et al., 2002; Leonard et al., 2002), it must be emphasized that different behavioral methodologies could account for these differences. Additionally, these findings could be very important clinically, as it suggests that nicotine may not be therapeutic in schizophrenia when auditory sensorimotor gating is tested over several days. Thus, past findings may be erroneous that have suggested that nicotine can be self-medication for reduction in PPI because of the lack of focus on persistent behavioral testing (Adler et al., 2002). As mentioned, a major issue is that the behavior of smoking cigarettes have been utilized in this clinical population and nicotine is not the only psychoactive substance in cigarettes. Finally, the findings of the changes in the RGS9 transcript suggests that the G-protein is more efficient in coupling to the dopamine D_2 receptor.

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