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The Effects of Nicotine Administration on Behavior and Markers of Brain Plasticity in a
Rodent Model of Psychosis

A dissertation

presented to

the faculty of the Department of Anatomy and Cell Biology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy in Biomedical Sciences

by

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ABSTRACT

The Effects of Nicotine Administration on Behavior and Markers of Brain Plasticity in a Rodent Model of Psychosis

by

Marla Perna

Schizophrenia affects about 1% of the population. A hallmark of the disorder is increased dopamine D2 receptor sensitivity in the brain. Studies have shown that schizophrenics smoke cigarettes at approximately 4 times the rate of the general population. It has been suggested that nicotine use is a form of self-medication for symptoms in schizophrenia. Smoking behaviors typically begin in adolescence. We assessed effects of nicotine on behavior and brain plasticity in an adolescent rodent model of schizophrenia with the goal of identifying targets for smoking cessation.

Methods: Rats were neonatally treated with quinpirole (a D2/D3 agonist) or saline and sensitized to 0.3, 0.5, or 0.7 mg/kg (free base) nicotine or saline, every other day for 9 days, and locomotor activity was recorded. After behavioral testing, animals demonstrating sensitization to 0.5mg/kg nicotine were surgically implanted with a guide cannula, aimed at the nucleus accumbens core. After recovery, animals underwent microdialysis and in vivo samples were collected every 20 minutes for 300 minutes. Postmortem brains from animals exposed to 0.5mg/kg nicotine or saline were dissected and the nucleus accumbens and dorsal striatum were analyzed for brain-derived neurotrophic factor (BDNF), phosphorylated cAMP response element binding protein

(pCREB), and glial-cell derived neurotrophic factor (GDNF), all proteins involved in neuronal plasticity. Results: Animals neonatally treated with quinpirole and administered nicotine showed robust increases in locomotor sensitization and a 400% increase in dopamine overflow from the accumbens core, which was greater than all other groups. Nicotine administration led to increased accumbal BDNF levels, which was enhanced by neonatal quinpirole pretreatment. GDNF levels were also increased in control animals given nicotine, which was attenuated to control levels by neonatal quinpirole. Finally, pCREB levels were robustly increased in animals neonatally treated with quinpirole, an effect that was partially attenuated by adolescent nicotine treatment. These data on pCREB suggest a possible biological marker of anhedonia. In conclusion, it is apparent that nicotine results in a robust increase in behavioral activity and changes in neural proteins of brain plasticity that may serve as possible pharmaceutical targets for smoking cessation in schizophrenia.

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CHAPTER 1

INTRODUCTION

Smoking is a major health concern for the general public as well as those with a comorbid mental disorder. Specifically, the prevalence of smoking in the schizophrenic population is 4 times the rate of smoking in the general population, yet the motivation for this increase in smoking behaviors is largely unfounded. It has been suggested that adolescent smoking may precede the onset and diagnosis of schizophrenia in this population [1] that leads to the importance of this stage in development of schizophrenia and comorbid nicotine use.

The most highly abused drug by schizophrenics is nicotine, which is the psychoactive ingredient in tobacco [1]. This set of experiments focused on the effects of nicotine on behavioral sensitization in a rodent model of schizophrenia and the relationship between adolescent nicotine exposure and proteins involved in brain plasticity. We used a rodent model of schizophrenia based on increased dopamine (DA) D₂ receptor function, a manipulation that has been shown to persist throughout the animal's lifetime [2]. The time period preceding diagnosis of schizophrenia, typically adolescence, is gaining recognition as a critical period for the initiation of smoking behaviors that may be precursors to or exacerbate the symptoms of schizophrenia. The ultimate goal of this research was to identify pharmacological targets for facilitating smoking cessation in schizophrenia.

Diagnoses and Characteristics of Schizophrenia

Schizophrenia affects approximately 1% of the population nationwide, with a slight increase in occurrence in males versus females [1,3,4]. Symptoms of

schizophrenia are dynamic in that they change over the course of the disease and over the lifetime of the patient. The symptoms of schizophrenia are typically classified into positive and negative categories. Positive symptoms include delusions, auditory and sensory hallucinations, and disorganized speech [5]. Negative symptoms include cognitive deficits in learning, memory, and attention, flattened affect (lack of expression of emotions), decreased motivation (anhedonia), and withdrawal from social situations [6]. There are 3 distinct and well defined stages of the disease that are not only important in diagnosing the disease but may also be important in treatment options for individuals suffering from schizophrenia and schizophrenia-like symptoms. The first stage is the *prodromal stage*, which typically occurs one year before diagnosis of the disease. Characteristics of the prodromal stage include withdrawal from social situations, decreased motivation (anhedonia), and flattened affect (reduced emotions) [5]. The second stage is the *active stage*, characterized by delusions, hallucinations, and psychotic episodes. The third stage is the *residual stage* in which symptomology commonly mimics the prodromal stage. Symptoms may be recognized as often as early childhood, while the disorder does not fully manifest until late adolescence [5].

Etiology of Schizophrenia

The etiology of schizophrenia has not been determined and several hypotheses describing aberrant neurotransmission in the brain are currently being investigated. One common thread in the overall function of the schizophrenic brain implies involvement of the DA system. In the late 1970s it was shown in postmortem brain tissue that nonmedicated schizophrenic humans had an increase in DA receptor sensitivity in several brain areas that differed significantly from control subjects [7]. The discovery of

the typical antipsychotics chlorpromazine in the mid 1950s and later haloperidol in the 1960s, which are both potent DA D₂ receptor antagonists, led to targeting of the DA D₂ receptor for treatment of schizophrenia. Currently, effective pharmacological treatment for the symptoms of schizophrenia relies on the drug's affinity for the D₂ receptor, and all current antipsychotic medications block the dopamine D₂ receptor with some affinity [8-10]. It is clear from past research that increased DA D₂ receptor function is implicated in the pathophysiology of schizophrenia, which was the primary focus for this study.

Over the past 30 years extensive research in the field of psychoses and schizophrenia has focused on DA function and dysfunction in the brain as it compares to neurotransmission in normal humans [7, 11, 12]. Early research suggested differences in limbic forebrain and striatal structures of the brain and decreased cortical volume [13]. Clinical observations pointed toward a role of DA in the psychotic brain because of 2 main findings. The first finding showed that acute, high doses of amphetamine were able to induce a schizophrenia-like state in normal individuals, suggesting increased DA function in schizophrenia [14]. The second finding revealed that effective neuroleptic medications had strong DA receptor antagonist effects on the brain [14]. Although the etiology of the schizophrenia remains inconclusive, knowledge of altered DA function is a major contributor to the understanding of the disorder in humans.

In addition to the role of DA function in schizophrenia, glutamate function in the brain has also been implicated in the pathophysiology of schizophrenia. This hypothesis suggests that the pathophysiology of schizophrenia may be due to glutamate hypofunction via decreased activation of the *N*-methyl-D-aspartate (NMDA) receptor in

this disorder [15, 16]. Glutamate and one of its receptor subtypes, the NMDA receptor, can directly and indirectly influence DA function in the brain [17]. Glutamate influences dopamine signaling in the pathways that connect the striatum and nucleus accumbens (Acb), and this circuitry controls learning, memory, and integration of cognition and motivation [18]. NMDA receptor antagonists such as phencyclidine (PCP) and ketamine decrease glutamate function [19] and secondarily increase DA function, which causes schizophrenia-like symptoms in humans [16]. Decreased gene expression for NMDA receptors has been shown in postmortem brain tissue of schizophrenic humans as compared to normal humans [20]. Administration of NMDA receptor antagonists to schizophrenics exacerbates preexisting symptoms and causes schizophrenia-like symptoms in normal humans. However, unlike DA receptor-targeted therapies for schizophrenia, glutamate receptor agonists fail to reduce symptoms of schizophrenia in humans.

Drug Stimulation of the Mesolimbic DA System

Dopaminergic cell bodies located in the ventral tegmental area [VTA] send major projections to brain areas including the basal ganglia, amygdala, and nucleus accumbens (Acb), and minor projections to the olfactory tubercle, hippocampus, and cerebral cortex [14]. An important dopamine projection that plays a major role in positive reinforcement and drug addiction originates from DA cell bodies in the VTA and projects to the nucleus accumbens and frontal cortex. The projection from the VTA to the Acb has been hypothesized to be the primary drug reinforcement pathway in the brain and is referred to as the mesolimbic dopamine pathway [21]. Behaviorally, stimulation of this pathway by abusive drugs increases self-administration, which is an accepted model of

drug seeking behavior in which the animal remotely administers a drug via an intravenous line or intracerebroventricular injection [22]; and locomotor activity, which is marked by increased locomotion in response to administration of a stimulant, and serves as a measurement for stimulant properties of drugs [23]. Additionally, conditioned place preference (CPP) serves as another measure for drug addiction by pairing the drug with a context in which the animal develops an association between a drug, such as nicotine, and the context in which the drug was administered [24]. Intra-cranial administration of DA receptor antagonists to the VTA or Acb blocks self-administration of reinforcing drugs, such as nicotine, cocaine, and amphetamine [24-26], which suggests participation of the mesolimbic DA pathway in reinforcement. Dopamine receptor antagonists block increases in locomotor activity to drugs of abuse, such as nicotine, and the associative effects of drugs that are mediated by the mesolimbic DA pathway [27-29]. Additionally, animals electrically self-stimulate the mesolimbic DA pathway via an electrode implanted in brain areas that modulate reward, such as the Acb and DS [30, 31]. Thus, it is apparent that the mesolimbic dopamine pathway is involved in mediating reinforcing effects of stimuli, and altered function of the mesolimbic pathway in schizophrenia may affect the reward potential of stimuli that act on the DA system.

Mechanisms of Drug Reward in the Brain

Stimulation of the mesolimbic pathway, which consists of major DA projections from the VTA to the Acb, leads to increased release of DA in the Acb [32, 33]. Increased DA function in the Acb has been shown to contribute to the rewarding and motivating properties of drugs of abuse, such as nicotine [34], and these findings will be discussed

briefly. Behaviorally, the activating and addictive properties of a given substance can be evaluated by increased locomotor sensitization, increased conditioned place preference, and increased self-administration of the drug using preclinical models. These behavioral tasks, mediated by increases in DA activity, are a result of increased DA release in the nucleus accumbens [Acb] [32, 33]. In contrast, decreased DA levels in this pathway lead to anhedonia, which can result from withdrawal from drugs of abuse or from a lack of DA function in the mesolimbic DA circuitry [35, 36]. It is apparent that the rewarding properties of drugs of abuse stem from activation of the mesolimbic dopamine system, resulting in increases in the dopamine response and pleasurable feelings that are associated with drug reward. Stimulation of DA neurons by administration of DA agonists leads to increased release of DA from the Acb; a relationship that is required in order to establish the rewarding effects of stimuli [37-39]. The discontinuation of drug use or withdrawal causes a dopamine-depleted state, which is used as a general anhedonia model [35, 40]. Behavioral studies have shown dopamine depleted animals exhibit higher self-administration of drugs of abuse than subjects with normal levels of dopamine, suggesting involvement of dopaminergic systems in the Acb and the reinforcing effects of addictive drugs [36]. Repeated exposure to DA agonists results in a heightened response of the DA system due to use of the drug over time, verified behaviorally by locomotor sensitization [41], and electrochemically by intracranial self-stimulation (ICSS) studies involving reward circuitry [32]. Behavioral studies have shown, for example, that sensitization to psychostimulants and other drugs of abuse is a causal link to repeated drug use [41]. Over time, repeated exposures to the same dose of a psychostimulant result in

increased behavioral response to the drug, also known as locomotor sensitization, or reverse tolerance, and this enhanced response is considered to be an indication of the addictive properties of stimuli, such as nicotine [41, 42].

Nicotine Action in the Brain

Nicotine is a member of the psychostimulant class of drugs [43]. Upon entering the blood stream, nicotine rapidly crosses the blood brain barrier and enters the brain where it acts as an agonist at endogenous nicotine acetylcholine receptors (nAChRs), which are located on many types of neurons in the brain, including, but not limited to acetylcholine, DA, and glutamate neurons [43]. To date, 17 genes encoding nAChRs have been identified in the brain and peripheral nervous system [44]. Nicotinic receptors are made up of a combination of alpha and beta subunits. Currently, 12 different subunits exist, consisting of 9 alpha ($\alpha 2$ - $\alpha 10$) and 3 beta ($\beta 2$ - $\beta 4$) subunits that have been identified and cloned in humans [44]. Nicotine acts on receptors in both the peripheral and central nervous system, with its greatest affinity for the receptors located in the central nervous system [45]. Two primary subtypes exist in the brain: the $\alpha 7$ nAChR and the $\alpha 4\beta 2$ nAChR. The latter contribute greater than 90% of the high affinity binding sites for nicotine in the rat brain [46, 47].

The amount of nicotine absorbed by the body depends on the route of administration (smoking, chewing gum, chewing tobacco, and snuff) and the dose of nicotine administered, which correlates with nicotine's effects on subsequent nAChR activation in the brain [46]. Endogenously, nAChRs bind to acetylcholine; however, nAChRs are presynaptically located on dopaminergic as well as glutamatergic neurons in the striatum and nucleus accumbens [48]. When these receptors are bound by

nicotine, calcium enters the presynaptic terminal and acts to increase the release of dopamine [48]. Increased DA release is indirectly affected by stimulation of $\alpha 7$ nAChRs, which are localized on glutamatergic neurons. When nAChRs on glutamate neurons are activated, glutamate is released and acts on DA neurons, which subsequently leads to DA release [49]. DA release is directly affected by stimulation of $\alpha 4\beta 2$ nAChRs, which are localized directly on dopaminergic neurons [48]. Additionally, nAChRs are present in the VTA, where their activation leads to excitation of DA neurons [50]. Thus, nicotine's agonist action produces either direct or indirect increases in dopaminergic function in brain areas of drug reward.

Nicotine Use in Schizophrenia

Comorbid drug use occurs with many psychological disorders including schizophrenia [51]. Findings have shown that the vast majority of schizophrenics are heavy smokers, and schizophrenics smoke 3 to 4 times more than the general smoking population [52]. And, nicotine is the most highly used addictive substance in schizophrenics. Schizophrenic smokers, through differences in inhalation patterns, extract more nicotine from a cigarette than do nonschizophrenic smokers, possibly increasing nicotine addiction [53]. Studies have shown that schizophrenics have increased serum levels of cotinine, a metabolite of nicotine, versus nonschizophrenic smokers [54]. Increased metabolites found in the serum of schizophrenics, which may again be due to an alteration in inhalation patterns, as compared to normal human smokers, that allows for larger amounts of nicotine to be extracted from each cigarette [54]. From a mechanistic standpoint, it has been suggested that a critical mediator of nicotine's effects in schizophrenia may be due to its action at the low affinity $\alpha 7$

receptor. This idea stems from analyses of postmortem brain tissues from human schizophrenics that have reported a significant decrease in hippocampal $\alpha 7$ nAChRs, which may possibly underlie cognitive impairment in this disorder [55, 56]. Because hippocampal $\alpha 7$ nAChRs are down-regulated in postmortem tissue in humans, Martin and Freedman have hypothesized nicotine use as a form of self-medication for cognitive impairment, possibly via alleviation of deficits in sensorimotor gating [57]. From a behavioral standpoint, increased nicotine action at the low affinity $\alpha 7$ receptor may alleviate cognitive deficits associated with low $\alpha 7$ binding in the hippocampus of schizophrenic humans [58]. Increased nicotine binding may provide the individual with increased selective attention, as shown by attenuation of PPI deficits in humans, and possibly alleviation of sensory dysfunction [54, 58, 59].

Consequences of Nicotine Use in Schizophrenia

Nicotine dependence in schizophrenics has been shown to decrease overall quality of life and shorten the lifespan of smoking schizophrenics by 20% [60]. For example, smoking may reduce blood levels of certain antipsychotic agents [45-47]. As a result, individuals with schizophrenia who smoke may require increased doses of antipsychotic medication [47, 48] that would lead to variable plasma concentrations of antipsychotic meds [49]. A known side effect of long-term antipsychotic medication administration, tardive dyskinesia (repetitive, involuntary body movements) has been linked to smoking behaviors in schizophrenics [45 51], which may result from higher doses of antipsychotics required in these patients [52]. Interestingly, research has shown that smoking behaviors are decreased in schizophrenics administered antipsychotic drugs than those who are not medicated [31]. In order to further examine

the relationship between antipsychotic medication and nicotine use on symptoms of schizophrenia, we first examined the effects of nicotine on behavior and neuronal adaptations in a rodent model of schizophrenia.

Modeling Psychoses in Rodents

These experiments focused on findings using a rodent model of schizophrenia. Rodent models of neurological disease are important and useful tools for studying components of specific diseases; however, modeling all aspects of a disorder is nearly impossible. Animal models can be useful for studying the etiology of a disorder, comparing treatments for a particular disease, or for development and discovery of new and more effective treatments for neurological disorders [61]. One disadvantage in using animal models is that they may not be able to predict the lifetime progression of a disorder. In most cases, animal models of neurological disease and dysfunction are used to model one or a few aspects of the disorder, whether they are behavioral, neurochemical, or neuropathological [62]. Findings from animals may be informative about the contribution of said behavioral or neurochemical abnormalities to the disease or disorder even if the entire disorder is not modeled in the animal [63]. Additionally, high genetic homology between rodents and humans allows for translation from animal to human. Additionally, this strengthens use of animal models for testing antipsychotic drugs, therefore supporting the use of animals to model neurological disease [64].

Pharmacological advances have increased management of schizophrenia in humans suffering from the disorder; however, the functional cause of the disease has yet to be determined. By modeling specific aspects of psychiatric disorders in an animal model, we are more likely to be able to understand the connection between the

neurological basis and the associated behavioral manifestations of the disorder [62].

Currently, over 60 rodent models of schizophrenia exist and are based on alterations in neurotransmitter function as well as various types of adult and neonatal brain lesions.

Criteria for Modeling Psychosis in Rodents

Attempts at modeling behavioral disorders in rodents have been made for decades, and few models, if any, encompass the full extent of its intended disorder. Because it is nearly impossible to model all aspects of a disorder, criteria for modeling disorders in animals have been suggested; however, to date, no stringent set of rules for modeling disorders in animals has been accepted. This is especially important with the translational focus that has developed at the National Institutes of Health (NIH). In 1977 McKinney proposed 4 criteria specific for validating animal models of disorder, as described by Tordjman and colleagues [62]. These criteria included similarity in behavior, inductive conditions, neurobiological mechanisms, and response to pharmacological treatment comparable to those seen in humans presenting the specific disorder [62]. Later, Robbins and Sahakian suggested 3 criteria, which were defined as face validity (behavioral similarity), construct validity (etiology or neurobiological similarities), and predictive validity (the ability to identify therapeutic medications for treatment of a disorder) to the actual human disorder [62, 66]. Finally, yet a third set of criteria for modeling human disorders in animals has been proposed that places each model into 3 distinct categories; homologous (face, construct, and etiologic validity), isomorphic (face validity), and predictive (predictive validity) [62]. *Homologous* models are rare and require identical symptomology as compared to humans over the time course of the disorder. As a result, many models displaying partial homology for a

disorder are used currently, such as those involving brain lesions. A model may be considered *isomorphic* if the animal displays identical symptoms to the human disorder, but the etiology of the disorder in the animal model and humans differ. One example of isomorphic modeling would be an animal model in which the human symptoms spontaneously occur, such as the spontaneously hypertensive rat, which has been used as a rodent model of Attention-Deficit Hyperactivity Disorder (ADHD). *Predictive* models can be used to screen the effectiveness of pharmacological treatments for a disorder [62]. Currently, there is no standard set of criteria for modeling disorders; however, each model has primary strengths and weaknesses. Several models exemplifying face, construct, or predictive validity will be discussed, including the neonatal quinpirole model that is the focus of the current study.

Because schizophrenia is known to result in a number of neurobiological abnormalities, there is no animal model that can effectively mimic all aspects of the disease process. Instead, individual aspects of the disease that parallel human symptoms and neurological abnormalities are modeled in an attempt to more fully understand the dysregulation that leads to psychoses [66]. A neural hallmark of schizophrenia is an increase in dopamine D₂ sensitivity, as all antipsychotics block the dopamine D₂ receptor with some affinity [67]. However, it is known that schizophrenia is a multi-faceted disorder with disruptions in a number of neurotransmitter systems. On the other hand, it is not known whether the changes in these neurotransmitter systems result from increases in D₂ sensitivity. This project is focused on a rodent model of psychosis, most applicable to schizophrenia, which was developed in Dr. Richard Kostrzewa's laboratory. In this model, quinpirole, a DA D₂/D₃ agonist, is given

neonatally resulting in an increase in dopamine D₂-like receptor sensitivity throughout the animal's lifetime without resulting in an increase in the number of D2 receptors [68]. The neonatal quinpirole model of schizophrenia is useful for assessing this disorder in rodents because it is a developmental model that is based on persistent DA receptor supersensitivity, unlike other models of schizophrenia, as detailed below.

Past Rodent Models of Schizophrenia

As previously stated, there are over 60 different animal models of schizophrenia in existence, and these models are derived in a variety of ways. Many models rely on a genetic mutation [64], alteration in prenatal development, such as maternal exposure to influenza [69] or other neuropathology, such as maternal separation and social isolation [70], all of which are consistent with schizophrenia. However, there are 2 types of models that are most often used when studying schizophrenia in rodents, which include acute drug exposure and neonatal lesions. These models and their strengths and weaknesses are described below.

The strength of past models is that they mimic positive and negative symptoms of schizophrenia in normal humans, and they alter neurotransmission in a fashion similar to the abnormalities observed in schizophrenia in humans. Administration of PCP or amphetamine results in psychotic-like symptoms in normal humans [71-73]; however major weaknesses exist in these models. PCP acts as an NMDA receptor antagonist, and a partial DA agonist, whereas amphetamine acts in part as a dopamine agonist. Acute, high dose administration of PCP or amphetamine results in changes in brain pharmacology that are consistent with schizophrenia; however these models do not produce a developmental change in the individual as is observed in human

schizophrenia [74]. Second, the PCP and amphetamine models do not produce long lasting neuronal adaptations that lead to psychotic symptoms.

Many models are based on lesions of specific brain areas to produce behaviors that parallel those exhibited by human schizophrenics. The most often used and validated rodent model of schizophrenia, the neonatal ventral hippocampal lesion model, relies on cell death in the ventral hippocampus to produce increased locomotor activity and changes in social interaction in rodents [75, 76]. This model was originally developed by Lipska and Weinberger [76, 77] and is based on hippocampal dysfunction, because schizophrenics show a significant increase in size of the lateral ventricles and significantly smaller hippocampi than normal subjects [78]. In this model, neonatal ventral hippocampal lesions are performed using ibotenic acid infusions on postnatal day 7, which results in abnormalities of numerous dopamine-related behaviors specific to schizophrenia in adulthood. For example, rats with neonatal lesions exhibit greater behavioral sensitization to cocaine and nicotine than control subjects, suggesting a higher susceptibility to psychostimulant abuse [79, 80]. Additionally, this model mimics the effects of changes in mRNA of DA and GABA markers in various regions of the brain that are also seen in human schizophrenics [81]. The neonatal lesion has shown a significant reduction in expression of dopamine transporter (DAT) mRNA and tyrosine hydroxylase (an enzyme involved in DA production) gene expression in the substantia nigra and VTA as adults. However, adult lesions do not show changes in expression of DAT or other DA related genes [82]. Behaviorally, neonatal hippocampal lesions produce several behavioral deficits consistent with schizophrenia, including hyperlocomotion, prepulse inhibition deficits, social withdrawal

and isolation [75], as well as cognitive impairment [83]. It is important to reiterate that there is no evidence of cell death in the hippocampus in human schizophrenics [78, 79, 84]; therefore, there are some weaknesses in this model that may not be accurate relative to the neuropathology in schizophrenia.

A Novel Rodent Model of Psychosis

Studies from a collaborating laboratory have shown that repeated quinpirole administration during neonatal development in rats produces a marked supersensitization of dopamine D₂-like receptors [2]. Increased D₂ receptor sensitivity is consistent with human schizophrenia [7], and all antipsychotic drugs target this receptor family with some affinity. Neonatal quinpirole treatment leads to D₂ supersensitization without changing DA receptor number, which is also consistent with human schizophrenia, as there has been no report of upregulation in DA D₂ receptors in humans [85]. Additionally, neonatal quinpirole treatment in rats results in supersensitization of the D₂/D₃ receptor that lasts throughout the animal's lifetime [2, 85]. Dopamine D₂ supersensitization is shown behaviorally through hyperlocomotion, increased vertical rearing, increased horizontal activity, and increased yawning in response to acute quinpirole treatment and dopamine-related behaviors [86].

Validation of the Neonatal Quinpirole Model of Schizophrenia

There are a number of consistencies between findings in schizophrenia in humans and the neonatal quinpirole model. Certain physical aspects of schizophrenia suggest that the disease is not due to neuropathology, or cell death in the brain, but due to a neurodevelopmental abnormality [74]. This model mimics developmental changes in the dopamine system present in human schizophrenia because neonatal quinpirole

treatment affects developmental stages of dopaminergic pathways. Findings using the neonatal quinpirole model have revealed several key behavioral and neurochemical deficits that are also observed in schizophrenics, which are detailed below.

Pharmacological Validation

A key hallmark of any rodent model of a disorder is that treatments commonly used to alleviate behavioral or neurochemical abnormalities in that disorder in humans have also been found to be effective in the rodent model. This laboratory reported that olanzapine treatment given twice daily for one month in adulthood alleviated cognitive deficits in Morris water maze performance produced by neonatal quinpirole treatment [87] consistent with human data [88]. Importantly, olanzapine alleviated the increase in yawning behavior, which is a D₂/D₃ receptor mediated event, in response to acute quinpirole in this model [87]. This demonstrates that increased D₂ sensitivity is likely responsible for these behavioral effects, and antipsychotic treatments are effective in alleviating these effects. *In vitro* analyses showed that decreases in hippocampal brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) produced by neonatal quinpirole were alleviated by olanzapine to control levels after an 8-day washout [87], also consistent with findings of this drug treatment in humans [89].

Altered Expression of Neurotrophic Factors in the Brain

Neonatal quinpirole treatments produce neurochemical abnormalities in adulthood that are consistent with observations made in human schizophrenics. Our laboratory has shown that neonatal quinpirole treatment produced significant decreases of hippocampal choline acetyltransferase (ChAT), BDNF, and NGF protein content compared to saline controls in both early postweanling and adult rats [90, 91].

Additionally, polymerase chain reaction (PCR) analyses from this laboratory have shown a significant decrease of NGF, BDNF, and ChAT expression in the hippocampus of animals neonatally treated with quinpirole. Human schizophrenics also demonstrate significant decreases in NGF [89] and BDNF protein [92, 93] as well as significant reductions in ChAT expression in the hippocampus [94, 95], Acb, and pontine tegmentum [92, 93], which are all consistent with this model.

Effects of Psychostimulants

Imaging studies have shown that amphetamine administration produces a large increase in dopamine overflow in the dorsal striatum of schizophrenics [96-98]. This lab and collaborators have shown that adult rats neonatally treated with quinpirole demonstrate more robust locomotor sensitization in response to adulthood amphetamine treatment [99], which also leads to a 500% increase in the release of striatal and accumbal dopamine overflow [100].

Deficits in RGS9 Protein in the Brain

RGS9 is a regulator of G-protein signaling at the dopamine D₂ receptor and RGS9 has been shown to be responsible for cessation of dopamine receptor activation due to action of a dopamine agonist. Postmortem analyses of schizophrenics have shown significant decreases of hippocampal *Rgs9* expression [101, 102]. Decreases in RGS9 protein activity are common in cases of dopaminergic hyperactivity [101]. We have shown through *in situ hybridization* that neonatal quinpirole treatment results in a robust significant decrease of *Rgs9* expression in the Acb, striatum, and frontal cortex [103].

Deficits in Prepulse Inhibition (PPI)

PPI provides an operational measure of sensorimotor gating, and has been shown across a number of studies to be disrupted in schizophrenia [104, 105]. PPI of the startle response refers to an attenuated response to a strong stimulus (pulse) when this is preceded shortly by a weak, nonstartling stimulus (prepulse). In fact, deficits in PPI have been suggested to be the most consistent behavioral hallmark of schizophrenia and to be the underlying mechanism of cognitive impairment in the disorder [106]. Neonatal quinpirole treatments have been shown to produce deficits in auditory sensorimotor gating using PPI. Unpublished data from our laboratory have shown that adult rats given neonatal quinpirole treatment demonstrated PPI deficits as compared to controls.

Cognitive Impairments

Neonatal quinpirole treatments have been shown to produce cognitive impairment that persists into adulthood [108]. Cognitive impairment is present in schizophrenia, and it has also been suggested that cognitive impairment is a core feature of the disorder [106]. This lab has shown in several studies that neonatal quinpirole treatment results in cognitive impairment in early postweanlings [92] and in adulthood [90, 107-109].

Dopamine, Schizophrenia, and Addiction: A Common Tie?

Interestingly, addiction to certain drugs, such as alcohol and amphetamine, as well as prenatal exposure to particular drugs, such as cocaine, has been shown to increase sensitivity of dopamine receptors. Addiction to nicotine is thought to be a result of nicotine's effects on the dopamine system [110]. The discovery of changes in

dopamine receptor function as a result of antipsychotic medication points to a shared underlying mechanism in both drug addiction and psychoses [111, 112]. A significantly higher incidence of psychostimulant abuse exists in the schizophrenic population as compared to the general population [59]. It has been estimated that up to 90% of the schizophrenic population smokes cigarettes, as compared to only 20% of the general population [55] and up to 77% of schizophrenics begin smoking before diagnosis of the disorder [112, 113]. Human schizophrenics exhibit differences in smoking patterns, rate of smoking, and inhalation patterns than normal smokers.

Schizophrenic smokers and normal smokers report similar reasons for nicotine use, including reduction of stress, anxiety, and agitation, and they report similar withdrawal symptoms. Comparatively, schizophrenics smoke more than normal humans, suggesting an increased motivation to smoke in the schizophrenic population [114]. Differences in smoking behaviors between these 2 populations led to the notion that schizophrenic smokers are using nicotine as a form of self-medication. As mentioned above, one possible explanation for using nicotine as a form of self-medication is to alleviate sensory gating abnormalities and attention deficits caused by the disorder [58, 113]. Additionally, some patients report an increase of psychiatric symptoms during withdrawal [35, 115], and these withdrawal symptoms are especially increased with antismoking agents [116]. Research has shown that use of certain neuroleptics causes an increase in cigarette smoking in schizophrenic patients due to dopamine D₂ receptor blockade. For example, the typical antipsychotic haloperidol, which is an especially potent dopamine D₂ receptor antagonist, was reported to increase smoking in normal nonschizophrenic smokers as compared to baseline [117].

It is believed that this is due to a decrease in function of dopamine receptor sites, namely the D₂ receptor, reinforcing nicotine intake in order to compensate for a lack of dopamine-related reward [118].

Reduction of Anhedonia: Self-Medicating With Nicotine?

One possible hypothesis for increased smoking in schizophrenia is that smoking is used to self-medicate a number of different symptoms. For example, a diminished reward system, due to neuroleptic antagonism of the DA system, has been suggested as the cause of apathy and lack of motivation characteristic of negative symptoms in schizophrenia [119]. Nicotine's ability to augment dopamine release is suggested to be a potential physiological antagonist to negative symptoms that may underlie the high prevalence of smoking in schizophrenics [120]. Nicotine alleviates negative symptoms, cognitive impairment, and behavioral abnormalities associated with schizophrenia [118]. Nicotine also reportedly reduces known side effects of antipsychotic treatment, such as lethargy and flattened affect, in treated individuals [113]. Corroborating the self-medication hypothesis of nicotine in schizophrenia, imaging studies in schizophrenics demonstrate a sensitized dopaminergic response to nicotine in brain reward areas [97], in agreement with past work on amphetamine and its effects on the dopamine system in schizophrenics [98]. In sum, increased DA activity, which mediates positive reinforcement, is the basis of this self-medication hypothesis.

Alongside the hypothesis of nicotine self-medication, it is also suggested that higher levels of smoking in schizophrenia may be attributed to other aspects of the disorder, such as personality traits. In general, smokers are more likely to exhibit neurotic traits and social alienation and less likely to exhibit achievement traits and

strong socioeconomic status [121]. Schizophrenics also fit this personality profile, with negative symptoms producing a general flat affect, and positive symptoms increasing paranoia and social alienation [4]. Together, this evidence strengthens the hypothesis of nicotine self-medication and an increased propensity to smoke in the schizophrenic population.

Effects of Nicotine on Rodent Behaviors

Increased dopamine release induced by nicotine has been hypothesized to play an important role in the reinforcing and locomotor stimulation effects of nicotine. For example, nicotine has been shown to increase locomotor sensitization when locally injected into the VTA [122]. The ability of nicotine to induce increased locomotion and also conditioned place preference [125] suggests that nicotine has psychostimulant and addictive properties [126]. The increase in behavioral response to nicotine has been shown through increased locomotion, increased vertical rearing behavior, as well as dopaminergic related behaviors such paw treading and grooming [38, 126, 127]. It is understood that other neurotransmitter systems are also involved in this phenomenon, but this may be attributed to modulation of other systems due to increased release of dopamine in the VTA-Acb pathway [121].

Adolescence: Psychostimulant Abuse and Psychoses

As suggested in the literature, smoking behaviors tend to begin in adolescence [128]. Although the diagnosis of schizophrenia does not occur until the late 20s, often the early phase of the disorder and occurrence of symptoms begins in adolescence [5]. Smoking in adolescence has been attributed to the severity of schizophrenia and have also been implicated as a precursor to this disease [1,129].

The DSM-IV states that the onset of schizophrenia typically occurs between the late teens and the mid-30s, thus, adolescent schizophrenia has not been adequately studied. It is apparent that some aspects of the disorder are observed during the early adolescent period before an individual presents with a full spectrum of schizophrenic symptoms [129], and schizophrenia is now considered to be a neurodevelopmental disorder [74] with its pathogenesis putatively stretching back to gestation and early childhood [130]. The use and abuse of alcohol, marijuana, psychostimulants, and other drugs are commonly found to be comorbid with psychiatric conditions in adolescents [131]. Weiser and colleagues [1] reported that in a large population of adolescents (14,000+), approximately 28.4% smoked at least one cigarette a day. Over a 4-16 year follow-up, it was found that adolescent smokers were at greater risk for schizophrenia, and the number of cigarettes smoked was associated with a significantly increased risk for schizophrenia. Compared to nonsmokers, adolescents who smoked at least 10 cigarettes/day were 2.28 times as likely to be hospitalized for schizophrenia than nonsmokers. Baker and colleagues [132] also reported that individuals diagnosed with psychosis began smoking daily at about 18 years of age, had higher levels of nicotine dependence, and demonstrated concurrent hazardous use of other drugs at a younger age.

One explanation for the increased propensity to use drugs in collaboration with the early emergence of symptoms leading to diagnosis of a disorder, such as schizophrenia, is that the adolescence period of development involves a rapidly changing environment in the brain in which the DA system development is highly vulnerable to changes induced by stimuli, such as nicotine [133]. During adolescence,

the DA system is dynamic, and this time period is marked by rapid changes in DA pathways [133]. The precise definition of adolescence has been debated in the literature but is described behaviorally by increased social behaviors and interactions as well as increased likelihood to engage in risky behaviors [134]. During this developmental period, adolescents are more vulnerable to the addictive properties of drugs of abuse, especially psychostimulants such as nicotine [127]. Recently, it has been suggested that psychostimulant abuse in adolescence may lead to sensitivity of the DA system. Subsequently, this may lead to manifestation of schizophrenia in adulthood [1]. It is also likely that adolescent psychostimulant abuse preceding diagnosis of disorder is an effect of the early stages of schizophrenia [127, 134, 135]. Sex differences in the effects of psychostimulants on adult rodents and humans have been shown [136, 137]; however, less is known about the effects on adolescent rodents and humans. Because adolescence is a crucial time for the development of neuronal pathways, especially DA pathways [133], it is important to emphasize the effects of psychostimulant abuse on neuronal adaptations in this population.

Neuronal Markers for Synaptic Plasticity

Drug use and drug addiction lead to changes in synaptic plasticity in the brain. Synaptic plasticity can be defined as a change in the communication between neurons in response to increases and decreases in use of a particular synaptic pathway, typically due to stimulation of the pathway via experience, learning, or the influence of drugs. The best-described mechanism of increased synaptic plasticity is NMDA receptor mediated, which leads to depolarization of the cell and subsequent calcium influx [138]. Calcium influx leads to initiation of the second mechanism, involving a signaling

cascade controlled by a second messenger system, leads to gene transcription and ultimately dendritic growth and changes in synaptic strength [138]. Under normal circumstances, bi-directional NMDA receptor activity contributes to homeostatic plasticity in the brain [138]. Homeostatic levels of NMDA receptor activation are altered by neuronal activation such as experience and drug abuse [139, 140]. Drugs of abuse can impact synaptic plasticity by increasing neurotransmission and expression of neurotrophic factors, such as BDNF [141]. Drugs of abuse also lead to increased gene expression mediated by activation of transcription factors such as cyclic-AMP response element binding protein (CREB) [142, 143].

Neurotrophic factors and transcription factors that regulate synaptic growth and plasticity are important in the pathology of schizophrenia and comorbid nicotine use mechanisms for several reasons. First, neurotransmitters and transcription factors contribute to the growth and maintenance of DA neurons. Second, they influence the development of DA neurons that may be crucial in the development of schizophrenia as well as increased use of psychostimulants, such as nicotine, in the schizophrenic population. Third, exposure to psychostimulants alters the expression of neurotransmitters and transcription factors, such as GDNF, NGF, BDNF, and CREB, leading to changes in synaptic plasticity [144-146].

Effects of Nicotine on Neuronal Markers of Plasticity

Abusive drugs, such as nicotine, lead to widespread changes in cellular activity in the brain. Changes in cellular activity in areas in the brain that modulate reward, especially the Acb, may serve as a cellular mechanism that underlies the motivation for repeated nicotine use [140, 143, 144]. For example, nicotine exposure in adult rats

leads to increased dendritic spine length and density in the Acb [147]; however, the specific mechanism underlying the connection between nicotine and increased spine density in Acb is still unknown [143]. In other brain areas, it has been shown that nicotine produces a significant increase in both NGF and BDNF protein in the hippocampus and in frontal cortex of adult rats [148]. Other psychostimulants, such as cocaine and amphetamine, produce significant increases in BDNF in the nucleus accumbens [149], suggesting that other DA agonists, such as nicotine, may also lead to changes in markers for synaptic plasticity in the Acb. In fact, one recent study showed increased BDNF levels in the Acb of mice treated with nicotine; however changes in BDNF and other markers for synaptic plasticity have not been examined in adolescent animals or in a rodent model of psychosis [87, 150, 151]. Behavioral studies have shown that adolescent and adult male rats exhibit conditioned place preference to nicotine [143, 152], which suggests associative properties and mild reinforcing effects of nicotine. Nicotine, cocaine, and alcohol induced CPP increases phosphorylated CREB levels in reward modulating areas of the brain, such as the lateral hypothalamus and VTA [153, 154], suggesting a connection between active drug seeking and plasticity of key reward areas in the brain.

GDNF

Midbrain GDNF, derived in the VTA, is expressed in the Acb and has been shown to specifically promote growth and survival of dopamine neurons in cell culture and is essential for full development of DA neurons [154]. All midbrain dopamine neurons express the receptor subunits for GDNF as well as mRNA for GDNF, except for the VTA that expresses the same receptor subunits but not mRNA for GDNF [156]. The

absence of GDNF mRNA in the VTA is due to retrograde signaling in the VTA and several experiments have shown that accumbal derived GDNF is retrogradely transported to the VTA and subsequently increases dopaminergic activity in the nucleus accumbens [157].

Developmentally, GDNF is found throughout the central nervous system where it promotes growth of dopamine neurons. In cell culture, GDNF stimulates neurite growth and differentiation and regulates the genes responsible for expression of TH and the dopamine transporter [158]. In the mature brain high levels are found in the nucleus accumbens and dorsal striatum where the function of GDNF is to aid in the integrity of dopamine neurons, functions, and pathways [155, 159, 160]. Dopamine firing is influenced by GDNF signaling, as shown by intra-VTA infusions of GDNF that result in increased spontaneous firing of VTA neurons as well as DA neurons [160]. Influence of GDNF in the ventral tegmental area as well as on the spontaneous firing of DA neurons has led to the study of GDNF's role in the actions of drugs of abuse as well as addiction.

GDNF and Addiction

GDNF has been suggested to be to be an “anti-addictive” protein because it reduces drug cravings and reward of abusive substances [157]. Infusion of GDNF into the VTA reduces reward and conversely, decreased GDNF increases drug reward and cravings, suggesting GDNF as a negative regulator of drug reward [162]. It is important to reiterate that drugs of abuse increase dopamine levels in the Acb and altered dopamine modulation underlies drug self-administration in animals [163]. Intra-VTA infusion of GDNF decreases conditioned place preference [162] for psychostimulants

and decreases self-administration of cocaine, ethanol, and sucrose in rats [164]. Meanwhile, a decrease in GDNF levels and subsequent signaling of GDNF in the VTA leads to increased behavioral sensitization, which is shown to be mediated by the nucleus accumbens. This indicates that normal GDNF function requires a feedback from the nucleus accumbens to the ventral tegmental area [165, 166]. Administration of GDNF into the VTA of mice reduced the rewarding effects of abusive drugs, and infusion of an antiaddiction drug, ibogain, into the VTA increases GDNF mRNA and protein levels, suggesting a role for GDNF in the addictive properties of drugs [164, 167]. Exogenous GDNF leads to decreased production of tyrosine hydroxylase and deltafosB [168-170], and anti-GDNF antibody administration into the VTA blocks these effects. Although there is an apparent relationship between GDNF and addiction, there are no known studies analyzing the effects of nicotine on GDNF, and no study has examined these factors in adolescence.

GDNF and Schizophrenia

The apparent role of GDNF in midbrain development and maintenance of DA neurons suggests a potential role for GDNF in the pathophysiology of psychosis in humans. Although little research has been done regarding a role for GDNF in schizophrenia, one study found that healthy human subjects possess a repeat in allele frequencies of the GDNF gene that was not found in schizophrenic subjects [171]; however, the aforementioned study is inconsistent with previous work showing no changes in allele frequencies for GDNF in schizophrenic patients [172]. Increased levels of serum GDNF have been reported in euthymic state (normal nondepressive and nonmanic state) bipolar patients compared to manic state patients and control subjects

[173], and another study showed decreased GDNF serum levels in depressed state and manic state patients as compared to control subjects, a deficit that was alleviated by pharmacological drug treatment [174], suggesting that GDNF is altered in some forms of psychoses and that chronic drug treatment may mask this evidence. Because most schizophrenic patients are medicated, altered GDNF levels may be more difficult to detect; however, animal models of increased psychoses, such as the neonatal quinpirole model may give insight to this potential mechanism.

cAMP Response Element Binding Protein (CREB)

CREB is a transcription factor that when activated regulates the transcription of specific genes. CREB is activated by a second messenger system that triggers an intracellular cascade of events that leads to activation of a protein kinase that translocates to the nucleus and activates CREB (by phosphorylation), which then binds to the CRE (cAMP regulated enhancer) binding site on DNA sequences in the nucleus and induces gene transcription [175]. The phosphorylated form of CREB (pCREB) has been shown to be necessary for long-term adaptations that lead to synaptic plasticity [176]. CREB regulates the transcription of several genes involved in neuroplasticity, including BDNF, tyrosine hydroxylase, and several neuropeptides [177]. Like BDNF, abusive drugs and stress also increase CREB activity, leading to studies demonstrating its importance in both processes. In terms of drug abuse, increased phosphorylated CREB levels in the Acb, mediated by exposure to drugs of abuse as well as environmental stress, leads to a profound effect on an animal's responsiveness to emotional stimuli [176, 178]. Importantly, viral vector-mediated elevations of CREB within the rat Acb *reduce* the rewarding effects of cocaine and morphine, as well as

sucrose [179, 180]. In fact, this CREB phenotype appears to reflect a generalized numbing of behavioral responses to emotional stimuli because animals with increased CREB function in the Acb also show reduced responses to a wide range of aversive conditions [176, 179]. Conversely, reduced CREB activity in the rat Acb, through viral vector-mediated expression of the dominant negative mutant mCREB, increases the rewarding effects of cocaine, morphine, and sucrose. It has been hypothesized that CREB is a key regulator of the reactivity of brain reward circuits that play a role in the processing of emotional stimuli [176, 179]. Most relevant to the current experiments, Carlezon and colleagues [176] suggest a sustained elevation of CREB activity in the Acb results in signs of anhedonia consistent with negative symptoms of schizophrenia. Interestingly, postmortem work in mood disorders, including schizophrenia has reported increased CREB in the prefrontal cortex [181], but the NAcc has not been analyzed in humans.

pCREB and Addiction

Drugs that increase dopamine function, including nicotine, lead to increased phosphorylation of CREB in the striatum, Acb, and VTA of adult animals and adolescent rats [143, 182, 183]. Nicotine results in CPP in adult rats and simultaneously, nicotine leads to increased pCREB protein levels and increased Fos expression in the VTA, Acb, and DS [184]. Additionally, administration of the nAChR antagonist, mecamylamine, abolishes increases in pCREB and Fos protein in response to conditioned place aversion (CPA) testing in adult rats [143]. In adolescent studies, rats with an increased reactivity to novel stimuli showed greater increases in locomotor activity due to nicotine exposure, which resulted in increased pCREB levels in the

striatum as compared to control adolescent rats [183]. These data suggest that an increased preference for nicotine and the increased locomotor activating properties of nicotine may lead to long lasting changes in synaptic plasticity in the brain, which could be controlled by phosphorylation of CREB. Importantly, the relationship between nicotine and pCREB in the Acb has not been established in schizophrenia. Findings may suggest a relationship between nicotine activation of the Acb and changes in pCREB in the self-medication hypothesis of nicotine use in schizophrenia.

The Anatomy of the Nucleus Accumbens and Dorsal Striatum

A brain area central to the rewarding effects of nicotine is the nucleus accumbens. Functionally and anatomically the Acb consists of 2 distinct regions: the core and shell. The Acb shell has been referred to as an anatomical extension of the amygdala and is involved in the motivated response of an organism or individual to a stimulus [185]. Acute drug administration in animals leads to increased DA release in the Acb shell, but chronic drug exposure in animals leads to a sensitized DA response from the Acb core [186]. Functionally, the Acb core is innervated by nuclei that process motor information, whereas the Acb shell may have a stronger association with the limbic system [187]. Anterograde and retrograde tracing studies have shown that the most pronounced differences between core and shell projections exist in regard to their connections to the hypothalamus and extended amygdala [188]. The core projects primarily to the entopeduncular nucleus including a part that invades the lateral hypothalamus, the shell projects diffusely throughout the rostrocaudal extent of the lateral hypothalamus as well as to the extended amygdala [188]. Histologically, the core is similar to the caudate-putamen in the basal ganglia, furthering its relationship to

locomotor activity, while the shell represents a transitional zone that seems to characterize most of the fringes of the striatal complex, where its interaction with the amygdala serves to integrate emotion and action [185, 189, 190]. The Acb core but not the shell has been implicated in conditioned dopamine release in psychostimulant locomotor sensitization [186], which leads to the importance of analyzing the DA response of the Acb core in the locomotor activating effects of nicotine in the neonatal quinpirole model.

Also important in the assessment of the psychomotor effects of nicotine and other psychostimulants is the DS. This area is responsible for integrating the DA input into the Acb while coordinating the effects of afferent information into behavior and sensorimotor responses [189]. Communication between the DS and Acb is important for the development of drug reinforcement, and function of the DS is necessary for the association of drug-paired cues [185]. Past work has shown that inactivation of the Acb core or the DS of rats decreases the responding for a cocaine-paired conditioned stimulus, demonstrating the importance of the dorsal striatum in the rewarding properties of psychostimulants [185].

In sum, it appears that there is a developmental change in the DA system of schizophrenic humans, leading to abnormal cellular communication. It is apparent that there is no single defined mechanism controlling schizophrenia, but rather a combination of behavioral and neurochemical abnormalities relative to the known symptoms that lead to the disorder. Schizophrenic humans exhibit differences in brain morphology, brain plasticity, DA function, and comorbid disorders, in addition to the positive and negative symptoms that characterize this group of individuals. To date,

there is no known underlying mechanism of the etiology of schizophrenia; however, it is apparent that many factors contribute to the aberrations between schizophrenic and normal humans. Current literature and knowledge of this disorder largely focuses on adulthood aspects of schizophrenia; however, far fewer experiments have focused on earlier manifestations of the disorder in humans. In order to gain a better perspective on the development of schizophrenia over the lifespan of the individual, it is important to begin examining the schizophrenia and schizophrenia-like symptoms in an adolescent model of the disorder. A better understanding of adolescent manifestations of schizophrenia and drug abuse mechanisms may lead to more effective therapeutic advancements in treatment and possibly attenuation of the severity of symptoms. This study will examine the effects of nicotine on the behaviors and biological markers of brain plasticity in the neonatal quinpirole model of psychosis, focusing on the early changes that occur during the adolescent period.

Hypotheses and Purpose of the Current Study

The purpose of the current study was to examine the effects of nicotine on brain plasticity markers and locomotor activity in an adolescent rodent model of psychosis. Past evidence from this laboratory showed that nicotine administration to adult and adolescent rats that were neonatally treated with quinpirole resulted in a more robust nicotine sensitization [126, 191]. It has been shown that a sensitized locomotor response to a repeated stimulus is a measure of the addictive and rewarding properties of a given stimulus. Several studies have shown that adolescent aged humans and rodents are reportedly more sensitive to the rewarding properties of drugs of abuse

[192], leading to the hypothesis that nicotine administration in adolescent animals will also result in a sensitized locomotor response.

Hypothesis I

Repeated nicotine administration will result in sex-differences in the sensitized locomotor response in adolescent animals in a dose dependent manner that will be enhanced by neonatal quinpirole.

Past data from this and other laboratories have shown that *in vivo* microdialysis of the Acb core and striatum in response to systemic amphetamine administration in adult animals neonatally treated with quinpirole leads to an increase in DA overflow versus animals given saline [99, 100]. The Acb is known to modulate reward as well as motivation, and the Acb core has especially been implicated in assessment of the behavioral response to rewarding stimuli [33]. These experiments lead to the hypothesis that nicotine administration would lead to increased DA release from the Acb core in adolescent animals that were administered nicotine, and that neonatal quinpirole would enhance this response.

Hypothesis II

It is hypothesized that in vivo microdialysis will result in increase DA overflow from the Acb core in response to systemic nicotine versus systemic saline in adolescent animals. Additionally, animals neonatally treated with quinpirole and given nicotine in adolescence will exhibit the highest accumbal DA response over all other treatment groups.

It has been shown previously that schizophrenic humans exhibit altered neuronal communication in the brain, a finding that has been attributed in altered expression and

protein levels of several neurotrophic factors and transcription factors, such as BDNF, NGF, and CREB [16, 193]. Aberrant expression of these factors has been shown in adults; however, there is little to no evidence in adolescent aged humans or rodents. It is hypothesized that neonatal quinpirole treatment in rodents will result in altered BDNF, GDNF, and pCREB protein levels in the Acb and striatum versus control animals. Additionally, these factors will further be influenced by adolescent nicotine administration.

Hypothesis IIIa

Adolescent nicotine treatment will result in increased BDNF protein levels in the Acb and striatum and that neonatal quinpirole treatment will enhance this effect.

Hypothesis IIIb

Adolescent nicotine treatment will result in decreased GDNF protein levels in the Acb and striatum and that neonatal quinpirole treatment will enhance this effect.

Hypothesis IIIc

Neonatal quinpirole treatment will result in an increase in pCREB protein levels in the Acb and striatum, which will be attenuated by adolescent nicotine administration.

CHAPTER 2

METHODS

Subjects

Adolescent animals were 35 days old and were randomly assigned to drug group by gender and neonatal treatment. Animals were assigned to drug groups (refer to Table 1) as follows (the first drug represents neonatal treatment and the second drug represents adolescent drug treatment): Quinpirole-Nicotine (QN), Quinpirole-Saline (QS), Saline-Nicotine (SN), and Saline-Saline (SS).

Table 1. Treatment groups for the current study

Gender	Neonatal tx	Adolescent tx	Code
Female	Saline	Saline	FSS
Female	Saline	Nicotine*	FSN
Female	Quinpirole	Saline	FQS
Female	Quinpirole	Nicotine*	FQN
Male	Saline	Saline	MSS
Male	Saline	Nicotine*	MSN
Male	Quinpirole	Saline	MQS
Male	Quinpirole	Nicotine*	MQN

* A dose response curve for nicotine was performed for behavioral sensitization.

Different groups of animals received 0.3, 0.5, or 0.7mg/kg nicotine during behavioral sensitization.

Research Design

For the proposed set of experiments, separate groups of animals were analyzed for each experiment. All procedures were performed within animal regulation, as approved by the Institutional Animal Care and Use Committee (IACUC) at East Tennessee State University. All animals were neonatally treated with either quinpirole or saline, and weaned at postnatal day 21. Separate groups of animals were administered 0.3, 0.5, or 0.7 mg/kg ip. nicotine (free base) injections during sensitization procedures. Only male animals that had been sensitized to the 0.5 mg/kg dose of nicotine were used for microdialysis and HPLC experiments because this dose produced the most robust sensitization curve, as shown in Figure 1 in the results section. Animals that were to be used for BDNF, GDNF, and pCREB analysis were only sensitized to the 0.5 mg/kg dose of nicotine and did not undergo microdialysis surgery.

Neonatal Drug Treatment. Animals were given a single daily intraperitoneal (i.p.) injection of either quinpirole (1mg/kg) or saline from postnatal day 1-21 (P1-21). All animals were administered 1mg/kg quinpirole based on body weight and were placed back into the home cage after injections. On P21, animals were weaned and socially housed (2-3 animals per cage) by gender.

Habituation to the Locomotor Arena. On P30-32, all animals were habituated to the locomotor testing arena. Each animal received an ip. injection of saline in the lower left quadrant of the abdomen and was placed in the home cage for 10 minutes before being placed in the testing arena. Animals were tested for 3 consecutive trials. This task provided a baseline activity measure and habituated the animal to the locomotor apparatus. Horizontal activity was measured by Anymaze software. AnyMaze

superimposes a grid of lines on to the locomotor arena. Each time the animal crossed one of the lines counted as one activity count, which was used as the primary dependent measure for behavioral sensitization.

Locomotor Sensitization. Following habituation, starting on P33, animals were given i.p. injections of nicotine tartarate (0.3, 0.5, or 0.7 mg/kg free base) or saline and placed back into the home cage until testing. Ten minutes after each injection, the animals were placed in the locomotor arena, and behavior was recorded for 10 minutes on each trial and horizontal activity counted was measured by Anymaze software. Testing was performed every other day for 16 days in all groups, from P33-49. The rationale for administering the drug every other day is based on the sensitization model of Pierce and Kalivas [41], which posits for behavioral sensitization to be measured properly, a day of no drug treatment must exist in between drug treatment days to allow for complete plasma clearance of the drug.

Guide Cannula Implant Surgical Procedure. For microdialysis, separate male and female animals treated with the 0.5 mg/kg nicotine dose (or saline) were analyzed. One day after sensitization testing, animals were fully anesthetized (50mg/kg ketamine, 10mg/kg xylazine, administered i.p. and sterile guide cannulae (Bioanalytical pin probe, Lafayette, IN, Part# MD-2200) were unilaterally implanted, using stereotaxic measurements. The guide cannulae were placed into the nucleus accumbens (+1.7mm AP, \pm 1.0mm ML, -6.4mm DV, measured from Bregma) and secured in place by inserting 3 mounting screws (PlasticsOne), which served to secure a dental acrylic cap. The acrylic cap was given ample drying time and incision was closed using sterile suture suture and wound clips. Cannulae placement was balanced across subjects

between left and right hemispheres to control for laterality effects. Postsurgery, animals were monitored during recovery, and, once ambulatory, returned to the home cage. Animals were allowed to recover for 2-10 days prior to microdialysis testing.

Microdialysis Procedure. On the day of microdialysis testing, microdialysis probes (2mM) were flushed for 1 h before use (1 μ l/min flow rate) with artificial cerebrospinal fluid (aCSF) (in mM: 145 NaCl, 2.8 KCl, 1.2 MgCl₂, 1.2 CaCl₂, 0.25 ascorbic acid, and 5.4 d-glucose, pH 6.5–7.0 adjusted with NaOH). Baseline samples were collected every 20 minutes for 2 hours (2 μ l/min flow rate) and served as a measure for basal dopamine levels for each individual animal. These samples were compared to samples taken from the same animal after systemic injection of saline or nicotine. All samples were collected into microcentrifuge tubes containing 50 μ l (70%) perchloric acid (used as a preservative and to acidify samples) and stored at -80°C until analysis by high performance liquid chromatography (HPLC).

Microdialysis Probe Placement. Postmortem brain tissues were analyzed for probe placement and subjects with misplaced cannulae were excluded from analyses. Probe placement was determined by blocking the tissue at the cannula insertion site and placement of the probe was verified, as has been shown previously [100].

High Performance Liquid Chromatography. Dialysate analysis of DA content was determined by HPLC. The HPLC method was used to separate, identify, and quantify compounds in dialysate liquid collected from microdialysis experiments. Dialysate components were compared to known concentrations of catecholamine standards containing DA, norepinephrine, and epinephrine (ESA catecholamine standard). A standard curve of concentrations for each component of the standard was determined

and optimized for the current study. Concentrations of samples were determined by comparisons of retention time between standard values and unknown samples. DA concentrations were recorded by measuring the area under the curve (AUC), as calculated by ESA software, for each sample tested. The HPLC (ESA-Coulochem) system consisted of a refrigerated microinjector and autosampler coupled to an electrochemical detection system. MD-TM mobile phase (0.15 M sodium phosphate, 2.24 mM sodium octanesulfonic acid, 0.94 mM EDTA and 10% acetonitrile, adjusted to pH 3.0 and filtered) was used at a flow rate of 0.6 ml/min at a gain of 10nA. The stationary phase consisted of a c-18 column (ESA model # 5101). To each sample, dihydroxybenzylamine (ESA) was added and served as an internal standard. DA was separated using an analytical cell and column for detecting monoamines and DA levels were quantified using an external standard curve. Retention time for DA was determined and area under the curve for each sample was calculated. From these data, the concentration of DA was compared to baseline levels of DA for each animal.

BDNF, GDNF, and pCREB ELISA Procedure. Twenty-four hours after testing, brains were removed, frozen in cold isopentane (-20°C), and stored at -80°C. Acb and dorsal striatum DS were dissected from the tissue samples and stored at -80°C until use. Individual samples were weighed and 250µl cold RIPA cell lysis buffer containing additional protease inhibitors (Sigma, p8340, p5726, p0044, PMSF) was added to each sample. All samples were homogenized via mortar and pestle method, sonicated using a tissue dismembranator, and centrifuged at 14,000xG for 20 minutes. Supernatants were removed and diluted 1:2 in sample buffer (according to the product specifications for each ELISA test) before use. Tissue sample homogenates were analyzed using a

BDNF or GDNF sandwich ELISA kit (Promega, Madison, WI) or a pCREB sandwich ELISA kit (R&D Systems, Minneapolis, MN). All steps and procedures for each ELISA were performed according to the manufacturer's instructions. For the assays, in brief, a 96 well plate (Nunc maxisorp, polystyrene) was coated with monoclonal antibody raised against the target protein. Nonspecific binding was blocked before addition of known and unknown amounts of each protein. The standard curve was prepared using serial dilutions of known amounts of BDNF, GDNF, and pCREB protein standards supplied by the manufacturer. The standards and samples were incubated at room temperature, followed by the addition of a polyclonal antibody raised against the target protein for each assay, which was then followed by the addition of anti-IgY horseradish peroxidase (HRP) conjugate. Visualization was achieved by adding 3,3',5,5'-tetramethylbenzidine (TMB) one solution and the reaction was stopped with 1N hydrochloric acid. All plates were read within 30 minutes of adding the stop solution using a Biorad plate reader with the absorbance set at 450nm.

CHAPTER 3

RESULTS

Horizontal Activity for Adolescent Males and Females

Horizontal activity for adolescent males and females is shown in Figure 1. A 4-way repeated measures ANOVA including the factors sex, neonatal drug treatment (saline, quinpirole), adolescent drug treatment (saline, 0.3, 0.5, 0.7 mg/kg nicotine), and day of treatment (days 1 and 9) revealed a significant main effect of neonatal drug treatment $F(1,92)=46.53, p<.01$ and adolescent drug treatment $F(3,92)=55.14, p<.01$. Analyses also revealed significant 2-way interactions of sex x adolescent drug treatment $F(3,92)=2.86, p<.04$, neonatal drug treatment x adolescent drug treatment $F(3,92)=9.82, p<.01$, adolescent drug treatment x day of treatment $F(3,92)=15.53, p<.01$, and a 3-way interaction of neonatal drug treatment x adolescent drug treatment x day of drug treatment $F(3,92)=3.26, p<.02$. Newman-Keuls post hoc analyses revealed both male and female Group QN7 demonstrated significantly higher locomotor activation at day 1 of treatment as compared to all other groups (indicated by **). On day 9 of treatment, only male Group QN7 demonstrated significantly higher activity counts than all other groups (indicated by ***), whereas female Groups QN5 and QN7 were equivalent but demonstrated significantly higher activity counts than all other groups (indicated by **). Further, at day 1, male Group SN7 demonstrated significantly higher activity counts compared to male Group SS, whereas female Groups SN5 and SN7 demonstrated higher activity counts compared to female Group SS at day 1 (indicated by *). Groups SN5 and SN7 both demonstrated higher levels of activity counts compared to Group SS at day 9, whereas female Groups SN3, SN5, and SN7 all

demonstrated significantly higher levels of activity counts than female Group SS at day 9, demonstrating a higher level of sensitivity to nicotine in adolescent females (indicated by *). The asterisk (*) and double asterisk (**) both indicate $p < .05$.

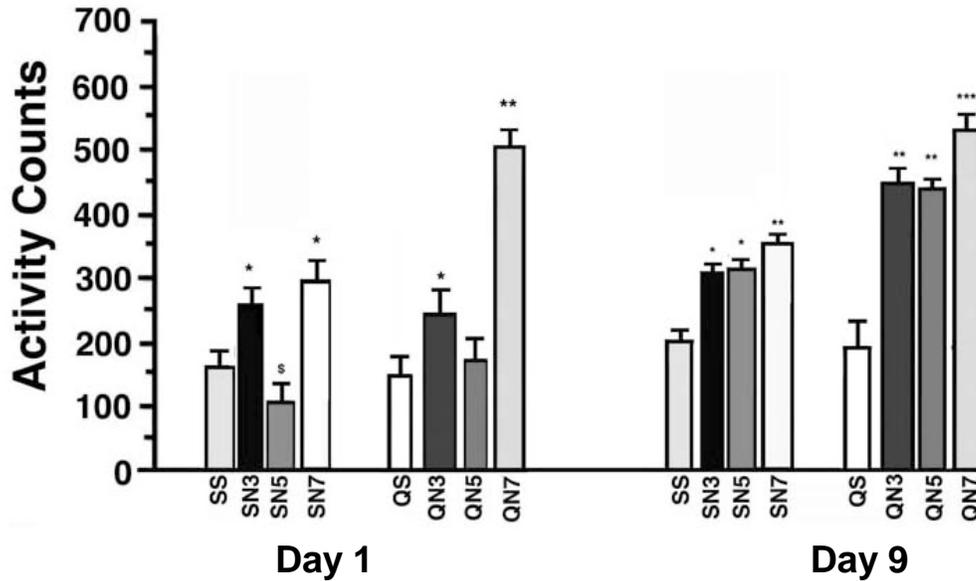


Figure 1a. Adolescent male locomotor sensitization to nicotine

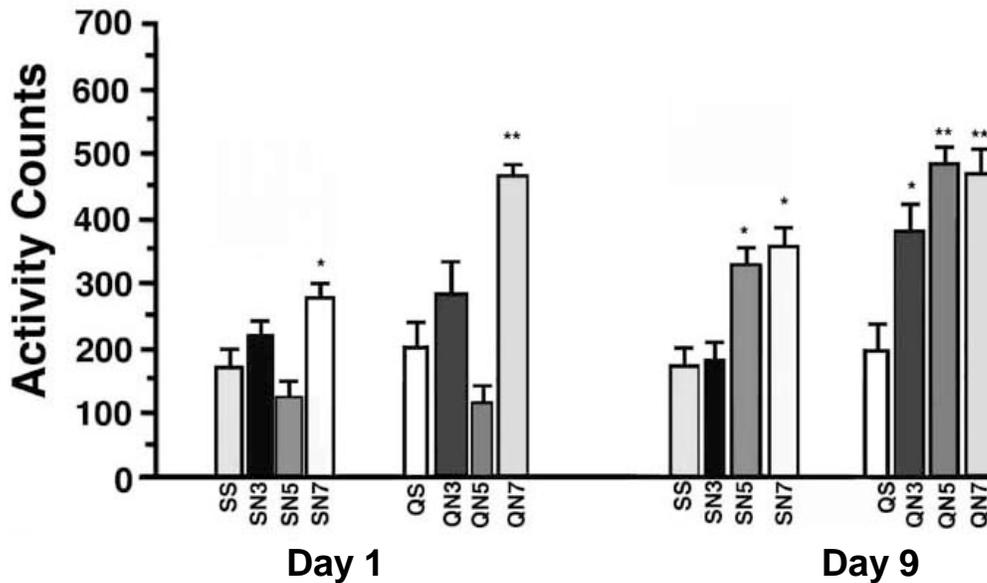


Figure 1b. Adolescent female locomotor sensitization to nicotine

Figure 1. Horizontal activity for adolescent males (top panel) and females (bottom panel)

Microdialysis Results

Adolescent males are presented with percentage of baseline as a function of group and time point in Figure 2. Baseline samples consisted of the first 3 samples taken from each animal in a drug free state. Samples after drug administration were compared to baseline samples from the same animal (within subject comparison). A 3-way ANOVA with neonatal drug treatment, adolescent drug treatment and time point (samples 1-9) revealed significant main effects of neonatal drug treatment $F(1,20)=13.0$, $p<.01$, adolescent drug treatment $F(1,20)=34.15$, $p<.01$, and significant interactions of neonatal drug treatment x adolescent drug treatment $F(1,20)=10.25$, $p.01$, neonatal drug treatment x time point $F(8,160) = 3.06$, $p<.01$, adolescent drug treatment x time point $F(8,160) = 6.10$, $p<.01$, and 3-way interaction of neonatal drug treatment x adolescent drug treatment x time point $F(8,160) = 3.79$, $p<.01$. Post hoc analyses revealed that Group QN demonstrated significantly higher dopamine levels as a percentage of baseline compared to all other groups at 80, 100, and 120 mins post nicotine treatment (indicated by **). Groups QN and SN were above controls at 60-160 min post nicotine treatment (indicated by *). The asterisk (*) and double asterisk (**) both indicate $p<.05$. Cumulative injections of nicotine (0.3mg/kg, 0.2mg/kg, 0.2mg/kg) or saline were given at time points 0, 20, 40, and are denoted by arrows below the graph.

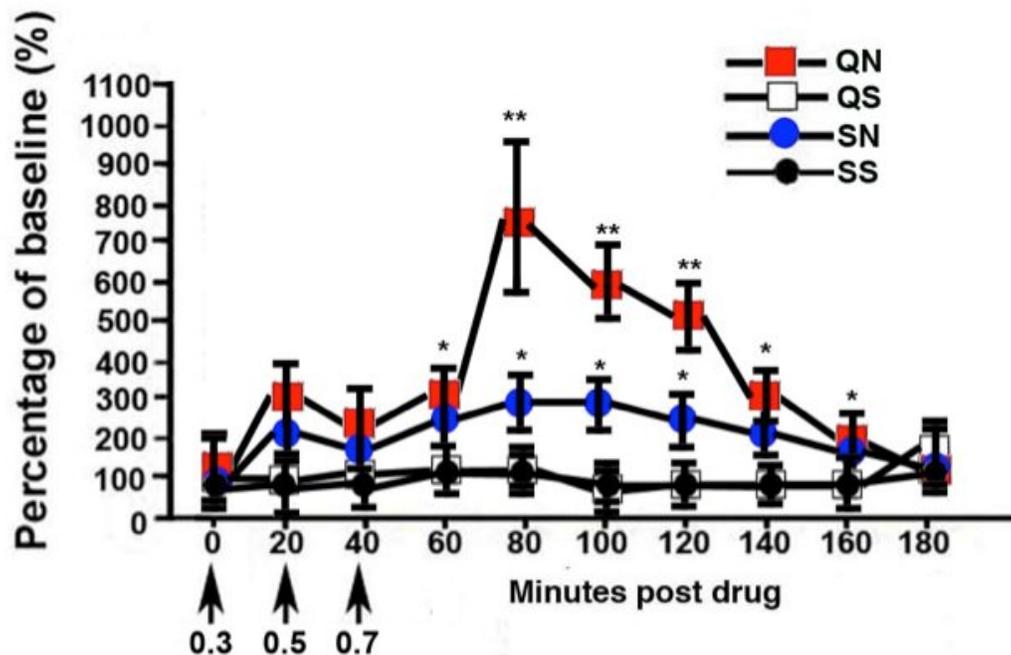


Figure 2. Microdialysis and HPLC results, DA levels for males only

Adolescent BDNF Results

BDNF is presented as a function of drug condition in Figure 3. In the Acb, no statistical effect including sex as a factor was significant, so this factor was dropped from the analysis. A 2 (neonatal drug treatment) x 2 (adolescent drug treatment) ANOVA for BDNF in the Acb (Fig. 3, right panel) revealed significant main effect of neonatal drug treatment $F(1,29) = 17.66, p < .001$, adolescent drug treatment $F(1,29) = 12.07, p < .002$, and a significant interaction of neonatal drug treatment x adolescent drug treatment $F(1,29) = 4.36, p < .047$. Group QN demonstrated a robust near three-fold increase in BDNF protein compared to controls (Group SS) as well as a significant better than 2-fold increase compared to Group S-N. In the dorsal striatum, no statistical effect including sex as a factor was significant, so this factor was dropped from the analysis. A 2 (neonatal drug treatment) x 2 (adolescent drug treatment) ANOVA for

BDNF in the dorsal striatum (Fig. 3, left panel) revealed a significant main effect of adolescent drug treatment $F(1,28) = 10.03, p < .004$. Nicotine produced a robust increase in BDNF in the dorsal striatum, but it was not enhanced by neonatal quinpirole. Newman Keuls post hoc comparisons revealed significant increases between Groups QN5 and SN5 compared to Group SS (control).

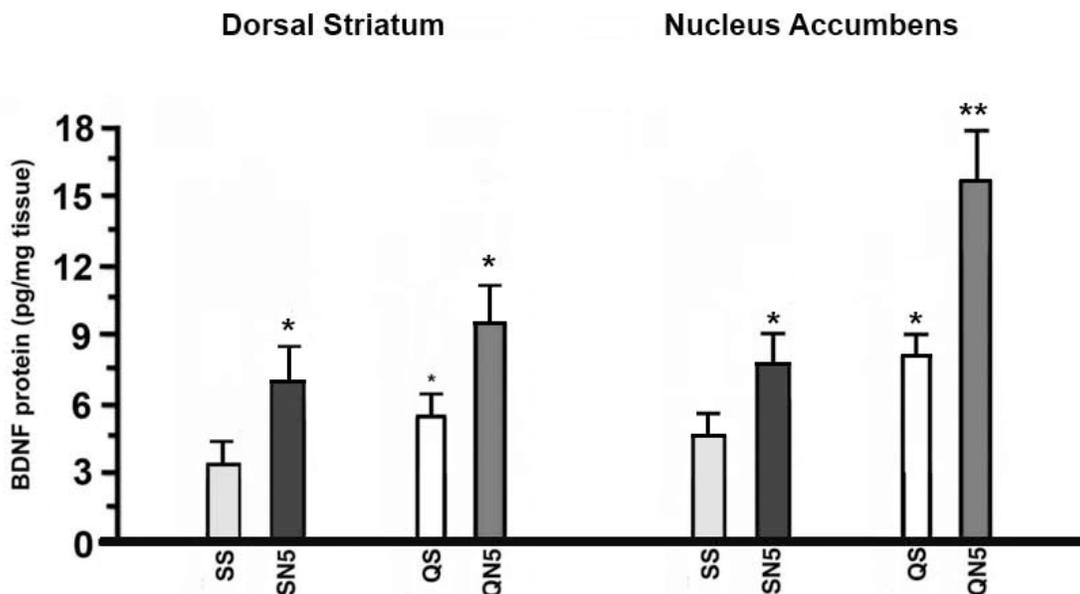


Figure 3. Adolescent BDNF protein content

Adolescent GDNF Results

GDNF results are presented in Figure 4 with GDNF presented as a function of drug condition. As with the BDNF results, there were no significant differences with sex as a factor so it was dropped from the analysis. In the Acb, a 2-way ANOVA revealed a significant neonatal drug treatment x adolescent drug treatment interaction $F(1,19) = 7.39, p < .015$ but no significant main effects of either factor. Newman Keuls post hoc tests revealed that Group S-N demonstrated a significant increase relative to all other

groups. Group S-N was increased relative to controls by 62%. In the striatum, a 2-way ANOVA revealed no significant main effects or interactions.

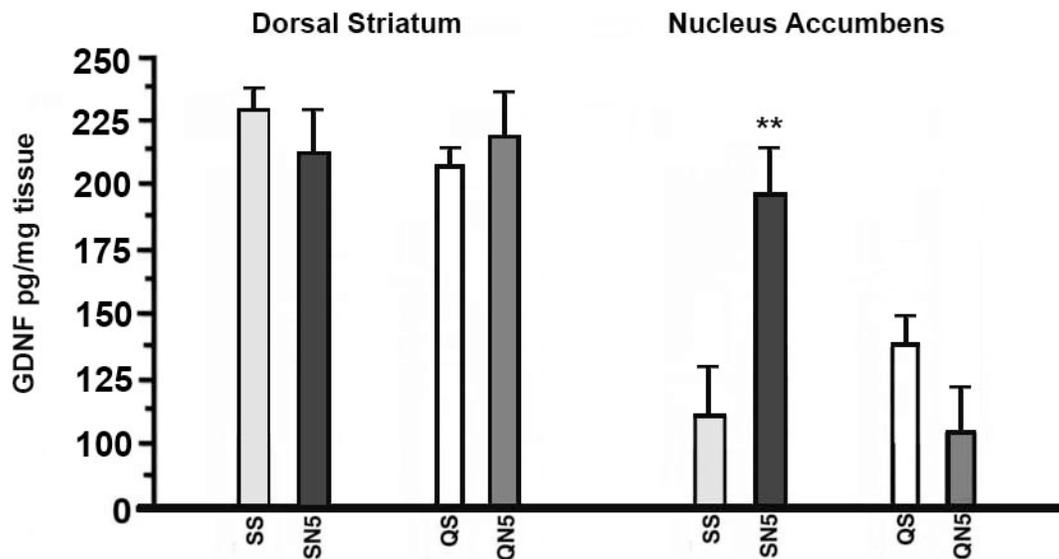


Figure 4. Adolescent GDNF protein content in DS and Acb

Adolescent pCREB Results

Phosphorylated CREB protein levels are presented as a function of drug condition. For the dorsal striatum, a 2-way ANOVA revealed a significant main effect of adolescent drug treatment $F(1,17) = 7.32, p < .019$. Nicotine produced a significant increase in p-CREB relative to controls. For the nucleus accumbens, a 2-way ANOVA revealed a significant main effect of neonatal drug treatment $F(1,17)=8.48, p < .012$ and a significant interaction of neonatal drug treatment x adolescent drug treatment $F(1,17)=5.11, p < .041$.

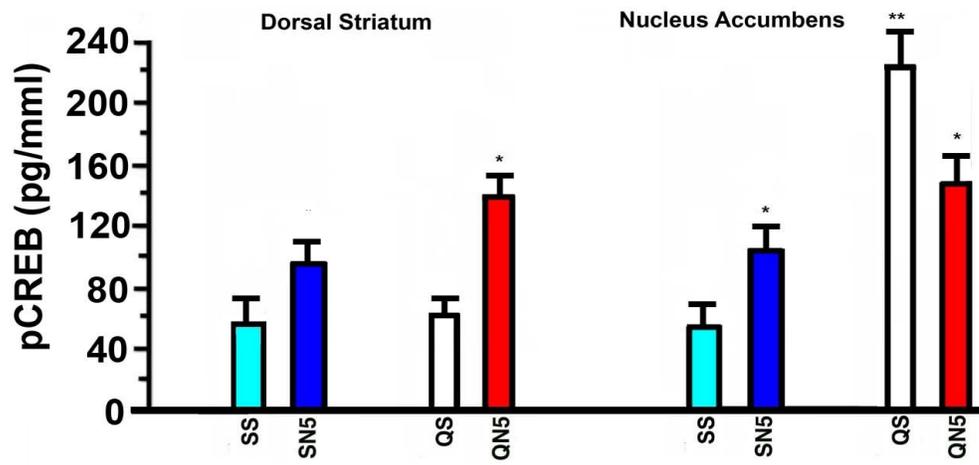


Figure 5. Adolescent pCREB protein content in DS and Acb

CHAPTER 4

DISCUSSION

Overall, these data demonstrate several significant behavioral and neurochemical changes as a result of neonatal quinpirole treatment on adolescent responses to nicotine. Behaviorally, as expected, adolescent animals neonatally treated with quinpirole demonstrated the highest increase in locomotor activity to nicotine administration. Additionally, *in vivo* microdialysis results revealed that adolescent animals neonatally treated with quinpirole showed a sensitized response in DA release in the Acb core as compared to control animals. Again, as expected, BDNF protein levels in the accumbens were increased by adolescent nicotine treatment and enhanced by neonatal quinpirole treatment. GDNF protein levels in the Acb were also increased by adolescent nicotine treatment; however, this effect was attenuated by neonatal quinpirole treatment. Finally, and possibly most importantly, pCREB protein were most significantly increased in the Acb by neonatal quinpirole treatment, an effect that was partially attenuated by adolescent nicotine administration in animals neonatally treated with quinpirole. This effect appears to be consistent with a robust increase in pCREB, which points to a possible state of anhedonia in these animals that may be reduced by nicotine [194]. Thus, this result would be consistent with the self-medication hypothesis of Glassman and colleagues [120] that nicotine may be working as a physiological antagonist to negative symptoms present in schizophrenia.

Sex Differences in Adolescent Locomotor Sensitization to Nicotine

Nicotine induced a significant increase in locomotor activity regardless of sex, a response that was enhanced by neonatal quinpirole treatment in both male and female

animals. Although animals that were treated with 0.7mg/kg nicotine showed the most robust increase in activity as compared to all other groups, this dose failed to result in a sensitized response to the activating effects of nicotine, in that animals did not demonstrate a significant increase in activity due to subsequent administration of nicotine. Females neonatally treated with quinpirole demonstrated equivalent levels of activity counts on day 9 with both the 0.5 and 0.7 mg/kg dose of nicotine, demonstrating a heightened sensitivity to lower nicotine doses as compared to males neonatally treated with quinpirole. The adolescent locomotor sensitization results reported here are similar to results previously reported in adults, in that neonatal quinpirole enhanced sensitization to nicotine. However, sex differences were more prominent in adults, in that a dose of 0.5 mg/kg free base was sufficient to produce stereotypy in female adult rats neonatally treated with quinpirole, and this effect was not observed in adolescent females [126]. In regard to sex differences in adolescent animals, females showed increased activity as compared to males at day 9 in response to repeated nicotine treatment, although this difference was relatively slight. In sum, the data support the first hypothesis stating that adolescent nicotine treatment would a) dose dependent result in sex differences in adolescent locomotor sensitization; b) the locomotor activating effects of nicotine would be enhanced by neonatal quinpirole treatment; c) the highest nicotine dose (0.7mg/kg) would result in the most robust increases in locomotor activity. The data, however, do not show that the adolescent animals were sensitized to the 0.7mg/kg dose of nicotine.

Data support past work from our laboratory showing increased locomotor activity in response to nicotine treatment in adult animals that were neonatally treated with

quinpirole [126]. Comparatively, adolescent animals were more sensitive to the activating effects of nicotine, especially females. Previous work from our laboratory demonstrated a lack of response to DA antagonists in males; however, in female animals, DA antagonism was able to block nicotine sensitization, suggesting that females are more sensitive to nicotine in adolescence [191].

Nicotine's Influence on Accumbal DA Levels

In summary, neonatal quinpirole enhanced dopamine overflow produced by nicotine in the nucleus accumbens core by 400% over controls given nicotine at the 80 min time point. The increase in dopamine overflow was evident at an earlier time point in animals neonatally treated with quinpirole, with significantly increased levels over controls 20 minutes before controls given nicotine and persisting for 40 minutes after dopamine levels in controls given nicotine had returned to baseline. Rats neonatally treated with saline and sensitized to nicotine demonstrated a 200% increase in dopamine compared to baseline at peak response. These data support the hypothesis that neonatal quinpirole treatment would result in the highest increase in DA release from the Acb in adolescent animals administered nicotine.

This finding is in support of the literature that suggests an enhanced DA response in the Acb to systemic nicotine administration [100]. Nicotine administration in animals neonatally treated with quinpirole resulted in a 400% increase in DA release in the Acb core, which may suggest that nicotine's rewarding properties are enhanced in these animals. As stated previously, repeated nicotine exposure leads to increased DA function in both the Acb core and shell, but increased DA overflow in the core of the Acb is more appropriately related to the expression of nicotine's effect on DA [187]. The

current findings support this work and, additionally, increases in locomotor activity, as shown by locomotor sensitization in Figure 1 show that nicotine induced increases in DA overflow correlate with an increase in locomotor activity.

Nicotine's Influence on Adolescent BDNF

From these analyses, it is clear that nicotine produced a significant increase in BDNF in both brain areas. In the Acb, nicotine administration lead to a 2-fold increase in BDNF levels in animals neonatally treated with quinpirole, as compared to all other groups. These results show that adolescent nicotine administration increased BDNF, and this appears to be most likely to be affected by neonatal quinpirole treatment. Additionally, these results are consistent with findings in human schizophrenic smokers. These results support the hypothesis that adolescent nicotine treatment would result in increased BDNF protein levels in the Acb and DS and that neonatal quinpirole treatment would enhance this effect.

Increased BDNF levels in the Acb and striatum are consistent with findings in the literature that suggest a role for BDNF in synaptic plasticity [33, 145]. Increased BDNF levels correlate with increased locomotor activity and increased motivation for drug taking behaviors. The current study showed a robust, 3-fold increase in BDNF levels in the Acb, and according to the literature, this increase suggests neuronal adaptations in the brain in response to nicotine exposure [140], which would lead to eventual nicotine addiction, and given that DA receptor supersensitivity leads to enhanced BDNF levels over control animals given nicotine, it is likely that the addictive properties of nicotine would be more pronounced in schizophrenic versus normal smokers.

Nicotine's influence on GDNF

Interestingly, GDNF protein levels were unchanged in all treatment groups in the dorsal striatum. In the Acb, adolescent nicotine administration resulted in a significant increase in GDNF protein levels above all other groups, but there was no significant effect of neonatal quinpirole treatment. Typically, GDNF levels are decreased in response to exposure to other drugs of abuse; however, nicotine's effects on GDNF have not been examined, especially in the context of the immature DA system of adolescents. These data do not support the hypothesis that adolescent nicotine treatment would decrease GDNF levels in the Acb and striatum, and that neonatal quinpirole treatment would lead to further decreases in GDNF protein levels.

In regard to nicotine's influence on GDNF protein levels in the striatum and Acb, these findings suggest that the nicotine administration in adolescence leads to the increased GDNF levels in the Acb. Findings in the literature, in which other DA agonists, such as cocaine were used [169], suggest that GDNF levels should be decreased in response to nicotine administration, contrary to the current experiments, demonstrating an increase in GDNF in the Acb. However, nicotine's effects on GDNF levels in the midbrain have not been studied. Additionally, immaturity and dynamic growth of the adolescent DA circuitry [137] may contribute to unexpected results. Neonatal quinpirole treatment did not enhance accumbal GDNF levels in response to nicotine, as seen in control animals given nicotine, which may suggest that increased sensitivity of the D₂ receptor leads to abnormal GDNF function in the brain. However, further exploration of this result is needed in order to better explain this relationship, such as examination of the effects of nicotine on GDNF levels in the adult brain.

Nicotine's influence on pCREB

Accumbal pCREB levels were significantly increased in animals neonatally treated with quinpirole, which may underlie anxiety and increased drug seeking behaviors. Nicotine administration in adolescent animals partially attenuated the increase in pCREB levels in the Acb, which would suggest that nicotine is decreasing stress levels in this group. These results support the hypothesis that neonatal quinpirole treatment will result in an increase in pCREB levels in the Acb and that adolescent nicotine administration would reduce this effect.

Data are consistent with work by Pascual et al. [130] that showed a lower dose [0.21 mg/kg free base] of nicotine as compared to the current study [0.5mg/kg free base] resulted in an increase of p-CREB in the Acb and DS. Interestingly, pCREB levels in animals neonatally treated with quinpirole were partially attenuated by nicotine treatment, as compared to animals neonatally treated with quinpirole that received saline in adolescence. This result suggests that pCREB protein levels may be a neurochemical marker for nicotine's use as a self-medication of negative symptoms in this model. As previously shown in rodents, stress induced by fear conditioning causes increased pCREB in the Acb [194]. In our model, we have shown that neonatal quinpirole treatment leads to a 4-fold increase in pCREB in the Acb of adolescent animals, and this increase is attenuated by adolescent nicotine treatment. These data suggest that nicotine administration in animals neonatally treated with quinpirole is rewarding because of its ability to decrease stress. Nicotine administration in adult and adolescent rats also leads to increased preference ratios in CPP and also increased pCREB in the VTA and Acb, suggesting that this increased preference is at least

partially modulated by changes in intracellular processes controlled by CREB activation. The increase of p-CREB is consistent with the notion put forth by Nestler and Carlezon [176] that robust and sustained increases in accumbal CREB may be an indicator for anhedonia in these animals, consistent with negative symptoms in schizophrenia.

BDNF, GDNF, and pCREB: Is There a Relationship?

Changes in the protein levels of BDNF, GDNF, and pCREB in relation to neonatal quinpirole treatment and in relation to adolescent nicotine administration have been demonstrated by this study; however, the real question may lie in how these factors are related to each other in the brain. Are these markers for plasticity related or is this finding just an interesting result within the manipulation of our model? This is certainly a question to be addressed in the future; however, it seems that the underlying connection between altered protein levels of BDNF, GDNF, and pCREB in the neonatal quinpirole model is largely due to modulation of the DA system. As these experiments have demonstrated, increased sensitivity of the DA receptor significantly affects the expression of BDNF, GDNF, and pCREB, all of which are suggested to play a role in brain plasticity. DA increases result in an increase in cell activity, leading to increased BDNF in response to nicotine, which has also been shown in human schizophrenia. Increases in stress are known to affect pCREB in animals and humans, and stress reduction is one common reason for smoking, stated by schizophrenic humans [122]. In our model, nicotine administration partially attenuated this increase in pCREB in the Acb, suggesting that nicotine may also be alleviating stress in this model, which would contribute to the motivation to increase nicotine intake, as shown by CPP studies from our lab, in which animals were more likely to spend time in a context that had been

previously paired with nicotine than a context that had been paired with saline on a drug free trial [195]. GDNF has been shown to be an inversely related to DA levels, suggesting that increases in DA would result in decreases in GDNF. Results from this study showed that nicotine administration increased GDNF in control animals; however, neonatal quinpirole treatment reduced that effect. However, it is not certain that BDNF, GDNF, and pCREB are influencing each other, because although CREB regulates BDNF, it is far downstream and regulates other proteins as well. GDNF has also been shown to be regulated by pCREB; however, there is very little supporting evidence for this relationship. There is also little evidence regarding the effects of nicotine on GDNF in the brain and no work has focused on adolescence.

Alpha7 nAChRs and schizophrenia

Postmortem, it has been reported that, schizophrenics have decreased $\alpha 7$ nAChR binding in the brain; however, the majority of this work has focused on the hippocampus [42]. There have not been any reports showing any change in $\alpha 4\beta 2$ nAChRs in schizophrenics. Behavioral studies have primarily focused on hippocampal $\alpha 7$ nAChRs as therapeutic targets for improving sensorimotor gating deficits that are known to exist in schizophrenics and have been suggested to be the root of cognitive deficits in the disorder [96]. In rodents, infusion of an $\alpha 7$ nAChR agonist into the Acb leads to increased nicotine self-administration in normal animals, which suggests that a decrease in function of $\alpha 7$ nAChRs contributes to increased motivation for nicotine [186]. In postmortem human studies, adult nonsmoking schizophrenics have decreased $\alpha 7$ nAChR mRNA and protein levels in the hippocampus than healthy nonsmokers, and smoking increases $\alpha 7$ nAChR mRNA and protein to control levels, suggesting abnormal

trafficking of these receptors [96]. However, this evidence in humans only pertains to a difference in $\alpha 7$ nAChR function in the hippocampus. Currently there is no evidence suggesting a change in $\alpha 7$ in brain areas mediating drug reward or on nAChRs in reward areas of the brain in schizophrenic smokers, and there are no data characterizing $\alpha 7$ nAChRs at earlier developmental stages in schizophrenics.

Interestingly, Tizabi and colleagues reported [70] that neonatal quinpirole resulted in a significant upregulation of $\alpha 7$ nAChRs in the striatum at P30 but no change in $\alpha 4\beta 2$ nAChRs in this same brain area. Obviously, P30 is near when nicotine treatment began in the present study. $\alpha 7$ receptors have been primarily identified as calcium (Ca^{+2}) channels. Calcium allows neurotransmitter release through entry into presynaptic terminals, causing depolarization of the cell, which allows for neurotransmitter release at the synapse. In the VTA and striatum, $\alpha 7$ receptors are localized on glutamatergic terminals, and the mechanism through which $\alpha 7$ nAChRs increase dopamine release in these areas has been attributed to glutamate function at the DA terminal [36], [39]. Research has shown that nicotine binding at the $\alpha 7$ receptor increases glutamate release, excites dopamine terminals, and leads to increased dopamine release. Glutamate induced DA release has been shown in vitro, as glutamate stimulates release of [^3H] dopamine from rat striatal slices [187], an effect that appears to be mediated by both types of glutamate receptor (AMPA/kainate and N-methyl-D-aspartate) present on dopaminergic nerve terminals [188]. Additionally, in vivo striatal infusion of NMDA [196-198] or AMPA [199] increases local release of dopamine. This is in contrast to the activation of $\alpha 4\beta 2$ nAChRs that has been shown to directly stimulate the release of dopamine from dopaminergic terminals in the neostriatum [48].

Therefore, it appears that $\alpha 7$ nAChRs indirectly influence dopaminergic activity in the striatum through a glutamatergic mechanism, whereas $\alpha 4\beta 2$ nAChRs directly influence dopamine release in the striatum through location of these receptors on dopaminergic terminals.

Therefore, in our model, one mechanism by which nicotine may be working is through the upregulation of $\alpha 7$ nAChRs. Although this has been shown only in the dorsal striatum, we assume the same phenomena is occurring in the Acb based on the fact that the source of dopamine for both areas is from the dopamine midbrain neurons. I hypothesize that nicotine, as an agonist to upregulated $\alpha 7$ nAChRs in rats neonatally treated with quinpirole, would produce an increase in dopamine overflow in these animals as compared to controls. This hypothesis would need to be verified with the use of a selective $\alpha 7$ antagonist administered before nicotine in rats neonatally treated with quinpirole. Our lab is currently working towards a series of experiments to analyze the role of nAChRs in this effect.

Ultimately, this project was designed to provide insight towards pharmaceutical targets for smoking cessation in schizophrenics. Given the data that have been presented, along with findings in the literature, it is apparent that intervention for smoking behaviors in adolescents is a clear target for reducing smoking, and potentially reducing the exacerbation of symptoms related to psychoses, such as schizophrenia. It is possible that modulation pCREB, GDNF, and BDNF would be potential targets for pharmaceutical therapies; however, this would require more in-depth experiments involving these markers for neuronal plasticity.

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