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Behavioral and Neurobiological Evidence of Epigenetic Transmission in the Neonatal Quinpirole Rodent Model of Schizophrenia

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by

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ABSTRACT

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Quinpirole is a dopamine D2 receptor agonist that if administered to rats from postnatal day (P)1-21 results in increased dopamine D2 receptor sensitivity throughout the animal's lifetime. This increase in receptor sensitivity is consistent with schizophrenia. This model has additional consistencies with human schizophrenia, including sensorimotor gating deficits, enhanced behavioral and neurobiological responses to nicotine, and protein alterations consistent with the disorder. In this study, a second generation of the neonatal quinpirole (NQ) rodent model was created to investigate if long term changes caused by NQ treatment would be passed to offspring. NQ treated rats were mated and their offspring left untreated. To investigate if dopamine D2 receptor hypersensitivity was transmitted from the first to the second generation of the model, yawning behavior was assayed after acute quinpirole treatment. Prepulse inhibition (PPI) is a test of sensorimotor gating, and PPI testing was performed on adolescent second generation rats. Behavioral sensitization and conditioned place preference to nicotine (0.5 mg/kg and 0.6 mg/kg respectively) were examined in adolescence in both generations of the model. Several neurobiological assays were performed in both nicotine naïve and animals sensitized to nicotine (0.5 mg/kg) in order to investigate consistencies with the NQ model, which has shown enhanced responses to nicotine. These include enzyme linked immunosorbent assays (ELISAs) for brain-

derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB), as well as quantitative PCR (qPCR) to quantify messenger RNA (mRNA) of regulator of G-protein signaling 9 (rgs9). Results indicated that second generation rats of NQ-treated rats demonstrated increased yawning behavior in response to acute quinpirole treatment. PPI deficits and enhanced behavioral responses to nicotine were also observed. Increased BDNF expression was observed in the nucleus accumbens following nicotine sensitization, consistent with past work in first generation NQ-treated rats. CREB expression was also increased in both generations of the model, an effect linked to alterations in PPI and other schizophrenia-like symptomology. *Rgs9* expression was generally unaltered in either generation of the model. This study provides basis for utilization of a second generation of the NQ model to study epigenetic influences in schizophrenia and drug abuse vulnerability.

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CHAPTER 1

INTRODUCTION

Schizophrenia is a neurological disorder that affects approximately 1% of the population (Kahn et al. 2015). The economic burden of schizophrenia in the United States was estimated to be greater than $155 billion in 2013 (Cloutier et al. 2016). In addition to great economic financial strain, schizophrenia also poses serious decreases to quality of life and lifespan of those affected. Patients diagnosed with schizophrenia experience an average lifespan which is 12-15 years shorter than the general population, due primarily to risk factors such as increased rate of smoking and obesity, likely caused by decreased exercise and poor diet (Saha et al. 2007). A heightened rate of suicide is exhibited in the population of patients diagnosed with schizophrenia, with the lifetime suicide risk rate estimated to be at 5% (Hor and Taylor 2010). These factors are largely influenced by the challenging nature of the treatment of schizophrenia, with approximately 30% of patients exhibiting treatment resistance (Meltzer 1997). The primary etiology is still largely unknown, which presents issues in identifying promising new treatment targets. Though many advancements in schizophrenia treatment have occurred over the past few decades, furthering understanding of the disease and exploration of additional treatment possibilities are necessary to improve the current landscape of schizophrenia.

Symptoms of Schizophrenia

Positive Symptoms

Schizophrenia is characterized by a multitude of symptoms, which typically begin to present in late adolescence (Hafner et al. 1994).These symptoms are typically

grouped into 3 categories: positive symptoms, negative symptoms, and cognitive symptoms. Positive symptoms describe experiences that those with schizophrenia have that do not occur in the healthy individuals. These symptoms are generally associated with a deviation in experience from reality. Positive symptoms include hallucinations, which can manifest in any of the sensory systems, as well as delusions (Picchioni and Murray 2007). These symptoms present challenges to preclinical schizophrenia researchers, as they are difficult (and potentially impossible) to detect and measure in animal models of schizophrenia which present symptomology from the other symptom categories. Therefore, it is difficult to assess whether novel treatments will alleviate positive symptoms until much later in the drug development process.

Negative Symptoms

Negative symptoms describe symptoms that are deficits in healthy behavior and response to stimuli. These symptoms include anhedonia, avolition, flattened affect, social withdrawal, and speech deficits (Foussias et al. 2015; Sarkar 2015). Unlike many positive symptoms, negative symptoms can be studied in animal models. Anhedonia has been demonstrated in animal models of schizophrenia utilizing sucrose preference (Turgeon and Hoge 2003; Baird et al. 2008). In these studies, when given free access to either a sucrose solution or water, the animals modelling schizophrenia showed decreased intake of sucrose solution compared to control animals, indicating that they experience decreased rewarding effect from the sucrose solution. Avolition has been studied in rodent models of schizophrenia using operant conditioning paradigms (Drew et al. 2007; Simpson et al. 2011). In these studies, rodents demonstrated decreased motivation to obtain reward via an operant conditioning paradigm requiring the rodents

to press a lever to receive reward. While deficits to speech cannot be evaluated in rodents as they lack use of discernable language, social deficits have been demonstrated via examination of ultrasonic vocalizations and grooming behaviors in rodent models of schizophrenia (Millan et al. 2014). Although the study of negative symptoms in preclinical models is viable and has produced heightened understanding of schizophrenia, current treatments are much less effective at treating negative symptoms compared to positive symptoms (Tandon et al. 2008a; Foussias et al. 2015).

Cognitive Symptoms

Cognitive symptoms of schizophrenia describe symptoms that include deficits to healthy cognitive function. Cognitive deficits are among the first symptoms to develop in an individual diagnosed with schizophrenia (Mesholam-Gately et al. 2009). Cognitive symptoms of schizophrenia include deficits to memory, attention, learning, and executive function (Tan 2009). Both working memory (Karlsgodt et al. 2007) and declarative memory (Karlsgodt et al. 2007) have been shown to be affected. These cognitive symptoms of schizophrenia have been extensively demonstrated by various models of the disease (Thacker et al. 2006; Miyamoto and Nitta 2014).

Prepulse inhibition (PPI) is a task that has been used to study cognitive performance in both individuals diagnosed with schizophrenia as well as rodent models of the disease (Light and Swerdlow 2014). PPI is a task that measures sensorimotor gating. Sensorimotor gating is the ability to use sensory stimuli to inhibit a motor response (Powell et al. 2012). Patients diagnosed with schizophrenia consistently demonstrate PPI deficits (Braff et al. 2001). However, PPI deficits are not limited to schizophrenia. Many other neurological conditions exhibit PPI deficits including

obsessive compulsive disorder, Tourette syndrome, and Huntington's disease (Swerdlow et al. 1995; Neal R. Swerdlow et al. 2001; Ahmari et al. 2012). PPI performance is affected by many brain regions and genes, but the PPI deficit phenotype appears to be heritable (N. R. Swerdlow et al. 2001; Greenwood et al. 2011). PPI has been shown to be critically useful in the screening process for models of schizophrenia. PPI has been demonstrated to correlate with various cognitive processes including working memory (Greenwood et al. 2013) and executive function (Bitsios et al. 2006; Swerdlow, Light, et al. 2006). Examination of PPI allows for the study of effectiveness of schizophrenia treatment. Atypical antipsychotics elevate PPI performance in schizophrenia patients as well as animal models (Swerdlow, Talledo, et al. 2006). Since PPI deficits have been demonstrated to be a hallmark of the disease (Light and Swerdlow 2014), their omission from any model brings validity to question of any such model.

Drug Abuse Vulnerability

A consistency of people diagnosed with schizophrenia is an increased rate of drug abuse, particularly with nicotine, alcohol, and cannabis (Barbee et al. 1989; Soyka et al. 1993; Salokangas et al. 2006; Featherstone and Siegel 2015). Focusing on nicotine, individuals diagnosed with schizophrenia are four times more likely to smoke tobacco, in which nicotine is the psychoactive ingredient in tobacco, than the healthy population (Featherstone and Siegel 2015). Additionally, individuals diagnosed with schizophrenia are more likely to be heavy smokers as well, which is defined as more than one and a half packs of cigarettes per day (Salokangas et al. 2006). While the cause of this increased rate in nicotine use is unknown, it has been suggested that

nicotine may provide relief from negative and cognitive symptoms associated with schizophrenia (LeDuc and Mittleman 1995). However, smoking produces several issues for individuals with schizophrenia. Smoking has been demonstrated to reduce blood levels of some antipsychotics, which play an important role in mitigating cognitive and positive symptoms of the disease (Jann et al. 1986; Carrillo et al. 2003). Smoking also introduces increased risk of lung and other cancers, respiratory illnesses, and cardiovascular diseases (Bonnie et al. 2015). Therefore, while nicotine may provide some therapeutic effects, the detriments to quality of life for individuals with schizophrenia necessitate research to alternatives and therapies that alleviate the rate of addiction in the affected population.

Dopamine Hypothesis of Schizophrenia

The exact cause of schizophrenia is still unknown. In fact, it is more likely that there is no specific singular cause for the symptomology of schizophrenia, and rather there are several contributing factors (Tandon et al. 2008b). However, a major underlying mechanism of the schizophrenia pathology is an increase in dopamine signaling and dysregulation, often referred to as the “Dopamine Hypothesis of Schizophrenia”. This hypothesis states that the behavioral symptoms of schizophrenia are caused by or related to dysfunction and/or hyperactivity in the dopamine systems in the brain (Howes and Kapur 2009). Some of the initial evidence for this theory was that antipsychotics that have proven effective at treating at least parts of the disease all antagonize dopamine D2 receptors (Seeman et al. 1976). There are three dopaminergic pathways in the brain which may explain and contribute to schizophrenia pathology. The mesolimbic pathway, or reward pathway, is the ventral tegmental area (VTA) to the

nucleus accumbens pathway. Dopamine alterations in this pathway may contribute to positive symptoms (Davis et al. 1991), increase incidence of drug addiction such as nicotine abuse in schizophrenia (Brown et al. 2012; Perna and Brown 2013; Lucatch et al. 2018), and also contribute to negative symptoms such as avolition (Salamone et al. 2007). The mesocortical pathway is the VTA to the prefrontal cortex pathway and is important in mediating cognitive processes and decision making, which are both deficient in schizophrenia (Floresco and Magyar 2006). The mesocortical pathway is also implicated in negative symptoms (Toda and Abi-Dargham 2007), due to its relationship to emotional reactivity. The nigrostriatal pathway is the pathway from the substantia nigra to the dorsal striatum. Dopamine signaling in the nigrostriatal pathway affects motor function as well as cognition (Graybiel 2008), and alterations to this pathway could explain learning deficits which are manifested in schizophrenia (Bach et al. 2008). The nigrostriatal pathway has also been implicated in the neurobiology of addiction (Wise 2009).

Dopamine D2 receptor antagonism is a focus of disease treatment (Abi-Dargham et al. 2000). It has been demonstrated that there are proportionately fewer unbound dopamine D2 receptors in individuals diagnosed with schizophrenia than the healthy population, indicating greater signaling through the dopamine D2 receptor in schizophrenia (Abi-Dargham et al. 2000). Dopamine D2 receptors have high-affinity and low-affinity states (Sibley et al. 1982). This increased binding of D2 receptors is indicative of an increased number of D2 receptors in the high-affinity state, or increased D2 receptor activity in this state, and this phenomenon has been demonstrated to occur

in patients diagnosed with schizophrenia as well as all relevant animal models (Seeman 2011).

Alternative Schizophrenia Hypotheses

While altered dopamine signaling is a hallmark of schizophrenia, there is disruption in other neurotransmitter systems in schizophrenia. For example, serotonin has been suggested to play a role in schizophrenia, affecting negative symptomology (Bleich et al. 1988). Additionally, antipsychotic medications in the atypical class also act on serotonin signaling, suggesting that serotonin dysfunction may play a role in the disease (Schmid et al. 2014). Post-mortem studies have also implicated serotonin receptors in schizophrenia pathology (Ngan et al. 2000).

Another neurotransmitter system affected in schizophrenia is glutamate. Many studies have shown hypoactive glutamatergic signaling contributes to schizophrenia's symptomology, and does so primarily through N-methyl D-aspartate (NMDA) receptor dysregulation (Stone et al. 2007). This hypothesis was in-part developed due to NMDA receptor antagonists resulting in similar symptoms to schizophrenia (Javitt 1987). This has also led to the development of schizophrenia models based on NMDA receptor antagonism (Olney et al. 1999; Lacroix et al. 2000). Though treatments targeting the glutamate system have previously been in development, no currently utilized treatments have been effective as NMDA receptor agonists. While it may seem that these hypotheses are competing, it is likely dysregulation of all three neurotransmitter systems contributes to schizophrenia symptomology (Howes et al. 2015). Therefore, while this dissertation project focuses primarily on dopaminergic contributions to schizophrenia,

research into either hypothesis is valuable to the continued understanding of the disease.

Epigenetic Transmission of Gene Regulation

Evidence of Epigenetic Contribution to Schizophrenia

Schizophrenia etiology is likely the combination of various genetic, environmental, and epigenetic factors, but the specifics of these factors are largely disputed. A key finding to support a mix of genetic and epigenetic factors is that monozygotic twins have a 41-65% concordance rate of schizophrenia compared to a 0-28% rate in dizygotic twins (Cardno and Gottesman 2000). Risk of the development of schizophrenia when just one parent has the disorder is around 13%, rising to 46% if both parents are diagnosed (Bromet et al. 2003). More than 700 genes have been researched as candidates for contributing to schizophrenia's etiology (Allen et al. 2008). Some of the most studied genes in relation to schizophrenia include brain-derived neurotrophic factor (BDNF), disrupted in schizophrenia 1 (DISC1), and dopamine receptor D2 (Farrell et al. 2015). Though heritability evidence as well as genetic studies demonstrate the importance of genetic variation in the etiology of schizophrenia, the lack of concordance between monozygotic twins suggest that the epigenome must also play an important role in the etiology of the disease. It is also important to note that heritability rates are not linked strictly to genetic variations, and that epigenetics also likely play an important role in heritability. In order to discuss how parts of the epigenome could be transmitted across generations, it is important to discuss the mechanisms affecting the epigenome and how these mechanisms ultimately conserved across generations.

Molecular Epigenetic Mechanisms

Epigenetics refers to molecular mechanisms which affect genetic expression without change to DNA sequence (Dupont et al. 2009). Many epigenetic mechanisms have been discovered, but chromatin remodeling is likely the most studied of these mechanisms. Chromatin is comprised of a complex of DNA and histones. Histones are proteins which form complexes to aid in compacting DNA (Strahl and Allis 2000). A useful, albeit imperfect, analogy to visualize histone function is that histone complexes act as a “spool”, with DNA acting as the “thread”. The tighter DNA is wound around the histone “spool”, the lower the transcription of genetic material interacting with the histone (Strahl and Allis 2000; Kramer 2013). Several molecular processes can lead to changes in the associations between DNA and histones, including DNA methylations (at CpG sites or not) and histone methylation, acetylation, or phosphorylation (Handy et al. 2011). Of these processes, the most studied pertaining to epigenetic inheritance is DNA methylation at CpG sites. This specific methylation event is most commonly correlated with downregulation of a gene via condensing of chromatin structure (i.e tightening of the DNA “thread” around the histone “spool”) (Strahl and Allis 2000).

Epigenetic Transmission Across Generations

The degree to which many epigenetic mechanisms controlling gene expression may be inherited by a future generation of offspring depends on the type of epigenetic mechanism and also on which parental sex is the contributor of a given epigenetic modifier. With regards to sex, maternal womb environment has large implication for epigenetic manipulation. A study of Dutch families following a famine found that adult offspring of parents who suffered through famine conditions experienced long lasting

metabolic issues, more attributed to maternal womb environment than epigenetically altered gamete genetic material (Lumey et al. 2007; Horsthemke 2018). However, epigenetic modifications to genetic material of germline cells also has implications for epigenetic transmission of gene regulation. Epigenome reprogramming is vitally important in the formation of germline cells. Epigenetic modifications are major contributors to cellular differentiation, and therefore are removed in order for germline cells to exhibit pluripotency (Tang et al. 2015). This is an effect which is seen in gametes of both parental sexes, but appears to be more dramatically exhibited in paternal germ-cells (Guo et al. 2014). Importantly, this reprogramming of gene methylation does not appear to affect the entire epigenome, as it appears some gene-groups linked to metabolic disorders such as diabetes and obesity, and neurological disorders such as schizophrenia are protected from this reprogramming event (Wei et al. 2014; Tang et al. 2015). Therefore, risk factors of disorders which are linked to epigenetic mechanisms such as DNA methylation have potential to be transmitted across generations.

Schizophrenia Treatment

There are two classes of antipsychotics that are directed towards treatment of schizophrenia. Second-generation antipsychotics, also called atypical antipsychotics, are the most commonly prescribed medications for schizophrenia. Atypical antipsychotics act primarily through dopamine D2 receptor antagonism, although they also bind serotonin receptor 5-HT2A receptors, along with a number of other pharmacological targets (Seeman 2002). While proven to be effective to alleviate positive symptoms of schizophrenia, atypical antipsychotics do little to improve negative

and cognitive symptoms, although they were designed to do so (Tandon et al. 2008a; Foussias et al. 2015). Of atypical psychotics, clozapine has proven the most commonly effective (Leucht et al. 2013). However, approximately 40-70% of individuals diagnosed with schizophrenia are resistant to treatment with clozapine (Taylor and Duncan-McConnell 2000).

Additionally, significant side-effects are a concern with atypical antipsychotics. Weight gain and associated metabolic conditions have been demonstrated to be a major side-effect of several antipsychotics (Allison et al. 1999; De Hert et al. 2012). Weight gain combined with inability to treat negative symptoms likely explain relatively low adherence to atypical antipsychotic prescriptions by patients diagnosed with schizophrenia. One study of 1493 patients diagnosed with schizophrenia across the US found that the 18-month adherence rate to atypical antipsychotics to be 26% (Leucht et al. 2013). Reasons given for discontinuation of use of medication largely revolved around both ineffectiveness of the medication to treat symptoms and intolerable side-effects. Significant side effects and low adherence rates to the most effective current treatments of schizophrenia highlight the need for novel treatment options and better understanding of the disease. To accomplish this, preclinical research utilizing effective modeling of schizophrenia is necessary.

Preclinical Models of Schizophrenia

Accurately modelling a complex neurological disorder such as schizophrenia presents many challenges. As the disease is recognized and diagnosed primarily based on behavior, cell lines are generally ineffective at modelling most aspects of schizophrenia. Another challenge is that the positive symptoms of schizophrenia, such

as hallucinations and delusions, cannot be studied in animal models. Consequently, models can only be validated based on negative and cognitive symptoms of the disease. More than 20 distinct models of schizophrenia have been developed and utilized to study behavioral, biochemical, genetic, and epigenetic aspects of schizophrenia (Carpenter and Koenig 2008). These models can be assigned to one of four categories: genetic models, lesion models, pharmacological models, and neurodevelopment models. Each of these model types, as well as individual models pertaining to each type, provide opportunities for studying particular aspects of schizophrenia, but not all models are suitable for studying every component of the disease.

Several genes have been suggested to play a role in schizophrenia etiology, most of which genes play a role in glutamatergic or dopamine systems, synaptogenesis, and neuronal plasticity (Harrison and Weinberger 2005). Mice with disrupted in schizophrenia 1 (DISC-1) knockout are one of the most common genetic-manipulation based models of schizophrenia (Jones et al. 2011). Notably, DISC-1 models altered ventricle size as well as PPI deficits which are reversed by antipsychotics (Clapcote et al. 2007; Jaaro-Peled et al. 2010). While DISC-1 knockouts and other genetic models can accurately reflect schizophrenia phenotype, they require genetic manipulations that could potentially be the consequence of epigenetic regulation in human schizophrenia. Therefore, the potential for study of epigenetic mechanisms in genetic manipulation models is limited.

Lesion models of schizophrenia involve the use of a brain lesion to produce schizophrenia-like phenotype. One commonly used lesion-induced model of

schizophrenia is the neonatal ventral hippocampal lesion model. This model is induced by acute bilateral injection of ibotenic acid, an endotoxin, into the ventral hippocampus of rats under anesthesia on post-natal day 7 (P7) (Lipska and Weinberger 1993). This treatment results in developmental changes in the rat, with key symptoms including enhanced dopamine D2 receptor sensitivity and impaired sensorimotor gaiting manifesting by P56 (Lipska and Weinberger 1993; Wan and Corbett 1997; Le Pen et al. 2000). This developmental progression closely models human schizophrenia, as human schizophrenia tends to present between the ages of 16-25 years, with P60 in rats generally being regarded as similar to a young adult (Sham et al. 1994). Relevant to this study, epigenetic alterations have been studied in this model and were demonstrated to play a large role in the induction of schizophrenia-like phenotype (Sandner et al. 2011). However, a critique of this model as well as other lesion-induced models of schizophrenia is that cell death is not present in schizophrenia (Harrison 1999; Harrison and Eastwood 2001).

Pharmacological models of schizophrenia rely on drug treatment, typically in adult rats, to induce a schizophrenia-like phenotype. Most commonly, phencyclidine (PCP) administration to rats either acutely or chronically to model symptoms seen in schizophrenia (Jones et al. 2011). PCP is an NMDA antagonist which elicits a psychosis response including hallucinations, paranoia, and catatonia (Bey and Patel 2007). It is for these schizophrenia-like symptoms following the use of this drug in otherwise healthy individuals that PCP was selected as a pharmacological agent for schizophrenia studies (Cohen et al. 1962). PPI deficits have been demonstrated in PCP-induced models, but these deficits diminish even in chronic models a soon following the cessation of PCP

treatment (Martinez et al. 1999). Pharmacological models do provide opportunity to study epigenetic related changes in expression levels of genes as well as investigate epigenetic mechanisms. However, since pharmacological models involve treatment typically being administered in adult rats, and effects in these models are often short-lived following drug treatment, the neurodevelopmental aspect of schizophrenia and its epigenetic components cannot be studied in models which do not incorporate a developmental period. This is important for the study of epigenetic mechanisms and their transmission, as schizophrenia is currently thought of as a neurodevelopmental disorder (Jones et al. 2011).

Neurodevelopmental models of schizophrenia are defined here as models which utilize gestational or neonatal treatments in order to produce schizophrenia-like phenotypes as a young rat ages. Neurodevelopmental models attempt to model not only the symptoms of the disease, but also the development of the disease. Gestational treatment with the microbial anti-inflammatory molecule protein (MAM) produces long-lasting schizophrenia-like symptoms in offspring of treated rats via DNA methylation (Matsumoto and Higa 1966; Lodge and Grace 2009; Jones et al. 2011). Given the developmental aspect, as well as long-lasting effects, neurodevelopmental models provide an opportunity to study epigenetics of schizophrenia as it relates to etiology, development, behavior, and pathology in a convergent way that many other model types do not.

Neonatal Quinpirole Model

The neonatal quinpirole (NQ) model is the neurodevelopmental model of schizophrenia utilized in this study. This model focuses on alteration to the dopamine

signaling system observed in human schizophrenia, primarily hypersensitive dopamine D2 receptors (Brown et al. 2012). Quinpirole is a selective dopamine D2/D3 receptor agonist which when administered to rats neonatally from P1 to P21 produces lifelong increase in the sensitivity of the dopamine D2 receptor (Kostrzewa et al. 2016). As previously mentioned, hypersensitivity of the dopamine D2 receptor is a biomolecular hallmark of the disorder and has been suggested to be the convergent point for many disrupted pathways observed in human schizophrenia (Seeman 2002). Behavioral validating findings within this model include cognitive impairments, PPI deficits, increased yawning in response to acute quinpirole in adulthood, and enhanced nicotine sensitization and conditioned place preference compared to neonatal saline-treated controls administered nicotine (Thacker et al. 2006; Brown et al. 2012; Maple et al. 2015; Peterson et al. 2017; Brown et al. 2018). Decreases in hippocampal nerve growth factor (NGF), choline acetyltransferase (ChAT), as well as a decrease in regulator of g-protein signaling 9 (rgs9) expression in dopamine terminal areas have all been observed (Brown et al. 2002; Maple et al. 2007; Brown et al. 2008), consistent with human schizophrenia (Bird et al. 1977; Perez-Polo et al. 1978; Seeman et al. 2007). These validations, as well as the neurodevelopmental nature of the model, provide the basis for utilizing this model to study epigenetic aspects, mechanisms, and transmission of schizophrenia as it pertains to dopamine D2 receptor hypersensitivity.

This model has been used to extensively study nicotine abuse and schizophrenia comorbidity. NQ treated rats exhibit a significantly increased response to nicotine in behavioral sensitization and conditioned place preference protocols (Brown et al. 2012; Perna and Brown 2013; Peterson et al. 2017; Brown et al. 2018). Upregulation of

neurotrophic factors BDNF and glial cell-derived neurotrophic factor (GDNF) following nicotine administration in these rats may partially explain the elevated response to nicotine in NQ rats (Perna and Brown 2013; Peterson et al. 2017; Brown et al. 2018). BDNF is a ubiquitously expressed protein which mediates neuroplasticity and has also been implicated in addiction development (Kowiański et al. 2018). GDNF is expressed primarily in areas of dopamine signaling, and is important in promoting dopamine neuron survival as well as neuroplasticity (Granholm et al. 2000; Lindholm et al. 2016). GDNF is also thought to mediate addiction (Kotyuk et al. 2016). Given this demonstrated enhanced response to nicotine in the NQ model, this model provides an opportunity to study how the epigenetic transmission of effects caused by dopamine D2 receptor hypersensitivity affects the potential for nicotine abuse in schizophrenia.

Study Purpose and Hypotheses

The purpose of this research project is to investigate the epigenetic transmission of dopamine hypersensitivity and associated schizophrenia-like effects utilizing the NQ rodent model of schizophrenia. Investigating if hypersensitivity at dopamine D2 receptors can be transmitted to offspring and how it occurs could lead to a better understanding of the elusive etiology of schizophrenia. Additionally, understanding this phenomenon could lead to identifications of novel treatment targets in schizophrenia, as well as highlight the function of dopamine hyperfunction as it pertains to both schizophrenia and drug abuse (specifically nicotine).To do this, rats neonatally treated with quinpirole (F0 rats) were mated, and the offspring, which were not treated neonatally, (F1 rats) were investigated for dopamine D2 receptor hypersensitivity. To investigate if both parents being treated neonatally was necessary for transmission of

dopamine D2 receptor hypersensitivity or if one parental-sex contributed more in epigenetic transmission, three different crosses were used to produce a second generation of the NQ rodent model of schizophrenia. One mating pair consisted of both parent rats being treated neonatally with quinpirole. The other two mating pairs consisted of one parent being treated neonatally with quinpirole, and the parent of the opposite sex being treated neonatally with saline.

To verify if dopamine D2 receptor hypersensitivity is passed along to F1 rats, a yawn test was performed on both generations of the NQ rodent model. The yawn test is a behavioral measure of dopamine D2 receptor sensitivity in which rats are injected with quinpirole, a dopamine D2 receptor agonist, and the number of times the rat yawns following this injection is counted for 1 hour. Yawning has been demonstrated to be D2-like receptor mediated (Cooper et al. 1989), and was shown to be elevated in neonatally quinpirole treated rats (Kostrzewa et al. 2016). Therefore, we hypothesized that yawning would be elevated following dopamine D2 receptor agonist injection in the neonatally untreated F1 rats with at least one neonatally treated quinpirole parent. PPI testing was used to measure sensorimotor gating in F0 and F1 rats. We hypothesized that like F0 neonatally treated quinpirole rats in previous studies (Maple et al. 2015), F1 with at least one neonatally treated quinpirole parent would exhibit PPI deficits compared to neonatal saline-treated controls.

We also investigated the effects nicotine would have on F1 rats. We hypothesized that F1 rats with at least one neonatally treated quinpirole parent would exhibit an enhanced response to nicotine via condition placed preference (CPP) and locomotor activity following a behavioral sensitization paradigm, as well as demonstrate

elevated BDNF expression following administration of nicotine. All of these nicotine-related effects have been demonstrated to be enhanced in F0 neonatally quinpirole treated rats (Brown et al. 2012; Peterson et al. 2017; Brown et al. 2018).

Potential mechanisms for hypersensitivity of dopamine D2 receptors in F1 rats were also investigated. Regulator of g-protein signaling 9 (rgs9) expression was investigated using qPCR. Rgs9-2, the splice isoform of rgs9 which is found in the brain, is a protein which binds to dopamine D2 receptors and prevents the receptor from achieving its high affinity state (Cabrera-Vera et al. 2004). Rgs9 has been shown to be downregulated in human schizophrenia (Seeman et al. 2007) and in the NQ rodent model (Maple et al. 2007). Therefore, we hypothesized that rgs9 RNA expression would be decreased in F1 rats with at least one neonatally treated quinpirole parent.

CREB protein expression was also investigated as a potential contributor to downstream effects of increased dopamine signaling. CREB primarily functions as a transcription factor, and is activated by phosphorylation by protein kinase A (PKA) as well as other kinases (Pláteník et al. 2005; Alberini 2009). CREB phosphorylation is linked to dopamine D2 receptor signaling (Yan et al. 1999). Alterations to CREB activity in the nucleus accumbens have been linked to PPI deficits (Culm et al. 2004) as well as blunted emotional response to aversive stimuli (Carlezon et al. 2005), both of which exist in schizophrenia. Therefore, an increase in CREB expression in the nucleus accumbens could be a downstream mechanism of epigenetically transmitted effects of dopamine D2 receptor hypersensitivity.

CHAPTER 2

MATERIALS AND METHODS

Animals and Materials

Subjects

A total of 311 animals were utilized in this study. All parts of this study were conducted using Sprague-Dawley rats. Postnatal day (P)0 indicates the day of birth of the subject animal. Subjects were weaned from their mother on P21. After weaning, subjects were socially housed, with 2-4 rats per cage, until the end of the experiment they were assigned to. All animals were housed on a 12 hour light and 12 hour dark cycle. Food and water were made available ad libitum throughout all experiments. All procedures for this study were approved by the East Tennessee State University Animal Care and Use Committee, which complies with the NIH Guide for the Care and Use of Animals.

Pharmacological Materials

This study utilized two drugs for treatment of rat subjects. Quinpirole HCl, a dopamine D2/D3 receptor agonist, and nicotine hydrogen tartarate were ordered from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Model Generation

Neonatal Treatment of F0 Rats

F0 rats in this study refer to rats which represent the first generation of rats being investigated. All F0 rats in this study were administered daily intraperitoneal (IP) injections of either saline (0.9% NaCl) or quinpirole HCL (1 mg/kg mixed in saline) from P1 to P21. F0 rats treated neonatally (N) with saline (S) are referred to in this study as

F0 NS. F0 rats treated neonatally (N) with quinpirole (Q) are referred to as F0 NQ. Neonatal treatment with quinpirole (NQ) produces rats with schizophrenia-like symptoms, detailed in Chapter 1. Rats treated neonatally with saline (NS) serve as controls. The purpose of saline injections is to control for stress of the injection.

Establishment of F1 Rats

In order to study the epigenetic transmission of schizophrenia-like symptoms conferred via NQ treatment, a second generation (F1) of rats were created. F1 rats are the offspring of F0 neonatally treated rats. F1 rats were not treated neonatally, but identical to F0 rats, were weaned from the female dam on P21. Three different parental mating-pair types were used to investigate the differences between epigenetic transmission contribution based on sex. The mating pair consisting of FQ and MQ was used to investigate epigenetic transmission when both parents were treated neonatally with quinpirole. Offspring from this mating-pair type will be referred to as F1 FQxMQ rats. The mating pair of FQ and MS was used to investigate the female parent rat's epigenetic contribution of transmission of schizophrenia-like symptoms. Offspring from this mating-pair type will be referred to as F1 FQxMS rats. The mating pair of FS and MQ was used to investigate the male parent rat's epigenetic contribution of transmission of schizophrenia-like symptoms. Offspring from this mating-pair type will be referred to as F1 FSxMQ rats.

It is possible that neonatal treatment may affect parental behavior, and that altered parental behavior could play a role in the development of a phenotype in F1 rats. To investigate this possibility, a subset of F1 rats were cross-fostered to a maternal

parent of the opposite neonatal treatment on P1. **Table 1** serves as a reference for the abbreviations used for all treatment groups throughout the study.

Table 1: Description of F0 and F1 Group Abbreviations

Abbreviation	Description
F0 NS	Rats treated neonatally with saline (0.9%, 1 mL/kg, IP)
F0 NQ	Rats treated neonatally with quinpirole (1 mg/kg, IP)
F1 FQxMQ	Untreated offspring of a female NQ and male NQ rat
F1 FQxMS	Untreated offspring of a female NQ and male NS rat
F1 FSxMQ	Untreated offspring of a female NS and male NQ rat
F1 FSxMS	Untreated offspring of a female NS and male NS rat

Experimental Methods

Yawn Test

To measure sensitivity of dopamine D2 receptors, F0 and F1 rats at age P60 were administered the yawn test as outlined by Kostrzewa and Brus (Kostrzewa and Brus 1991). Rats were given an IP injection of quinpirole (0.1 mg/kg) and immediately observed for 60 min. During this time, the number of times the rat yawned was counted. Yawning has been demonstrated to be a dopamine D2 receptor mediated event (Cooper et al. 1989), and increased sensitivity at the dopamine D2 receptor would manifest in increased yawning following administration of a dopamine D2 agonist such as quinpirole.

Auditory Prepulse Inhibition

The methods used for PPI testing in this was performed based on previous work from our laboratory (Maple et al. 2015). Beginning at P44, F0 and F1 rats were administered PPI testing sessions once daily for 3 days. The testing apparatus consisted of an tall stainless-steel dome with hard plastic flooring (8 cm tall x 11 cm wide x 15 cm long) mounted on top of a weight-sensing platform, all of which was contained within a sound attenuating chamber (28 cm tall x 30 cm wide x 36 cm long). The software utilized was Kinder Scientific Startle Monitor (Poway, CA, USA). After being placed into the testing apparatus, a 5 min habituation period was administered, in which only a background 70 decibels (dB) of white noise is played through a speaker inside of the sound attenuating chamber. After habituation, rats were administered 60 total trials during the daily session. For the duration of the 60-trial session, the 70 decibels (dB) of background white noise was played. Three different trial types made up the 60 total trials, including 20 *pulse* trials, 30 *prepulse* trials, and 10 *no stimulus* trials played in a semi-random order with randomized inter-trial intervals ranging between 8 and 22 seconds (s). A *pulse* trial consisted of a 120 dB auditory pulse followed by a 250 ms measuring window in which the startle response of the subject was measured in Newtons (N). A *prepulse* trial consisted of a prepulse, followed 100 ms later by a 120 dB pulse and 250 ms measuring window. The 30 *prepulse* trials were made up of 3 different *prepulse* trial types of differing prepulse dB levels, including 10 of each of the following: 73 dB *prepulse* trials, 76 dB *prepulse* trials, and 82 dB *prepulse* trials. A *no stimulus* trial consists of a 250 ms measuring window with no preceding prepulse or pulse. Each PPI data session including habituation period lasted for a duration of

approximately 20 min. Data collected through the Startle Monitor software was averaged over 3 days of testing.

Nicotine Behavioral Sensitization

At P30, female F0 and F1 rats were placed into a locomotor arena (black plexiglass cube with open top side; 91 cm tall x 91 cm wide x 91 long) 15 min following an IP saline injection for 5 min once daily for 3 days to habituate them to the testing arena. Starting the following day (P33), rats were IP injected with either saline or nicotine (0.5 mg/kg). Rats were placed into the locomotor arena 15 min later where they remained for 10 min. During the 10 min period in the locomotor arena, the total distance traveled by the rat was recorded as a measure of overall activity. Activity was measured in distance (m) and was calculated as a difference in activity from Day 1 to Day 9 of nicotine treatment. This saline or nicotine injection followed by locomotor measurement was administered every other day over 18 days with nine total drug administrations. Animal tracking was performed using AnyMaze behavioral scanning software (Stoelting Co., Wood Dale, IL, USA).

Nicotine Conditioned Place Preference (CPP)

A three chambered CPP apparatus (61 cm deep x 33 cm wide x 81 cm long) was utilized for this test. The three chambers all have identical dimensions (33 cm wide x 27 cm long) but have distinct tactile and visual contexts. The middle chamber flooring is made of Plexiglas, with walls painted black. One of the two outside chambers flooring is made of chicken-wire style metal and has vertical black and white stripes. The other outside chamber flooring is made of thin metal rebar and has horizontal black and white

stripes. The chambers are separated by removable dividers, which were removed to allow free movement among the three chambers.

F0 and F1 rats were administered two initial preference tests on P41 and P42 to determine initial context preference. During this initial preference test, rats were IP injected with saline, followed by a 10 min waiting period. Rats were then placed into the apparatus with dividers removed and allowed to freely explore the apparatus for 10 min. Mean time spent in the vertical and horizontal-striped contexts across the two testing days determined initial preference. Conditioning began on P43 and was conducted every day through P50, with the dividers placed into the apparatus. Conditioning consisted of 2 trials per day, an AM session and a PM session. During the AM session, all subjects were IP administered saline and placed into their assigned unpaired context for a 10 min trial. During the PM session, all rats were administered an IP injection of saline or nicotine (0.6 mg/kg) and placed into the paired context for a 10 min trial. The assignment of each context was selected so that the paired context for each subject was the opposite context of their initial preference. If there was no robust initial preference, the paired context was randomly chosen. On P51, rats were again tested for context preference as they were on P41 and P42, termed the post-conditioning preference test. The percent difference between the post-conditioning context preference and the pre-conditioning context preference was used as the measure of CPP. Animal tracking was performed using AnyMaze behavioral scanning software (Stoelting Co., Wood Dale, IL, USA).

Brain-derived Neurotrophic Factor Enzyme-linked Immunosorbent Assay (ELISA)

Approximately 24 hours following the final nicotine injection of the behavioral sensitization paradigm, female rats from all five treatment groups were decapitated and their brain tissue collected. Brains were immediately frozen in isopentane and stored at -80°C. Nucleus accumbens tissue was dissected from each brain and stored at -80°C. A BDNF ELISA kit was purchased from Promega, Inc. (Madison, WI). The provided protocol for this kit was followed, as described in a previous publication from our laboratory (Perna and Brown 2013). In brief, nucleus accumbens tissue was homogenized in RIPA cell lysis buffer (150 mM NaCl, 50 mM Tris-HCl, 1.0% NP-40, 0.5% Sodium deoxycholate and0.1% SDS) containing protease and phosphatase inhibitors P5726, P8340, and P0044 (Sigma-Aldrich, St. Louis, MO) using a sonic tissue homogenizer (Fisher Dismembrator 500). Tissue homogenates were centrifuged at 14,000g for 20 minutes and the supernatants extracted. A 96-well polystyrene plate was then coated with monoclonal anti-BDNF antibody using a carbonate coating buffer and concentration of antibody specified in the protocol provided by Promega. The coated plate was incubated overnight. A BDNF standard curve was prepared using BDNF protein isolate following dilution recommendations in the provided protocol. After washing with the supplied wash buffer, samples and the prepared BDNF standard were then added to the 96-well plate and incubated for 2 hours. Polyclonal BDNF antibody was then added, followed by an additional 2-hour incubation. Anti-IgY horseradish peroxidase conjugate was then added to the 96-well plate. TMB one solution was then added to the 96 wells and incubated for 10 minutes, followed by the addition of 1N hydrochloric acid to stop the reaction and allow for measurement to occur at 450 nm. To

quantify protein amount, optical density was measured using a Bio-Tek ELx 800 microplate reader (Winooski, VT).

Rgs9 Quantitative Polymerase Chain Reaction (qPCR)

F0 and F1 rats were decapitated at P60. A subset of these rats received 6 days of once daily nicotine injections (0.5 mg/kg, IP), with the final injection being 24 hours prior to decapitation at P60. Brains frozen in isopentane and stored at -80°C. Nucleus accumbens tissue was dissected from each brain and again stored at -80°C. Brains were then homogenized by hand in TRIzol (ThermoFisher, Waltham, MA) using a small pestle. Total RNA was then isolated from the tissue homogenates using a Direct-zol RNA MicroPrep Kit (Zymo Research, Irvine, CA). The quality of the total RNA isolate was analyzed using a Bioanalzyer RNA 6000 Nanochip (Agilent Technologies, Santa Clara, CA) and an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA). Total RNA samples deemed low quality were thrown out. From the RNA isolates, double stranded cDNA was made via reverse transcription using a Superscript III kit (Life Technologies, Grand Island, NY). Using this cDNA, quantitative PCR (qPCR) was used to quantify mRNA expression of 3 genes: *rgs9*, *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*), and *hypoxanthine guanine phosphoribosyl transferase* (*HPRT*). Reactions for each sample contained 5 ng of cDNA template, SYBR Green Master Mix (Qiagen, Valencia, CA), the primer specific to which gene was being assayed, and molecular grade water. All samples were run in triplicate, and a standard curve for each gene was run in duplicate. qPCR of sample reactions were performed and analyzed using Stratagene Mx300P (Alligent Technologies, Santa Clara, CA). The reaction protocol for SYBR green system were single incubations of 50°C for 2 minutes

and 95°C for 15 minutes followed with 35 cycles of 95°C for 30 seconds, primer specific annealing temperature for 30 seconds, and 72°C for one minute. A melting curve was performed for each primer to ensure a single product was produced. Expression of *rgs9* was normalized to the expression of housekeeping genes *GAPDH* and *HPRT* for each rat. This normalization controls for loading errors and variation among samples. Fold change in the normalized *rgs9* expression was calculated compared to the normalized *rgs9* expression of F0 NS rats using the method described by Livak and Schmittgen (Livak and Schmittgen 2001).

CREB Protein ELISA

At P60, rats from F0 NS, F0 NQ, and F1 FQxMQ treatment groups that were behaviorally tested on PPI were decapitated and their brain tissue collected. Brains were immediately frozen in isopentane and stored at -80°C. Nucleus accumbens tissue was dissected from each brain and again stored at -80°C. A CREB ELISA kit was purchased from MyBioSource (San Diego, CA, USA). The protocol provided with this kit was closely followed. In brief, nucleus accumbens tissue was homogenized in RIPA cell lysis buffer containing protease and phosphatase inhibitors as previously stated using a sonic tissue homogenizer. Tissue was centrifuged, and the supernatant was collected. CREB protein provided for use as a standard was serial diluted as described in the protocol. The standard dilutions and samples were added to the pre-coated 96-well plate. After incubation and wash, the provided Detection Reagent A was applied. The plate was then incubated and washed again, and Detection Reagent B was applied. Following another incubation and was, TMB was applied to the plate. The provided stop solution was applied to the plate after a brief incubation. The plate was then read using

a plate reader at a wavelength of 450 nm. Data was converted to pg/mg using the standard curve calculated using the standard dilutions.

Statistical Analysis

All statistical analysis was performed using SPSS. In all experiments, ANOVA was the primary statistical test performed. If an ANOVA test returned a significant group effect, a Newman-Keuls post hoc test was performed in order to make individual comparisons of groups within a given experiment. For all statistical comparison tests, a p-value of less than 0.05 was used as the indicator of a statistically significance difference between groups. Unless noted otherwise, an asterisk (*) in figures denotes a statistically significant difference in that group compared to controls.

CHAPTER 3

RESULTS

Transmission of Schizophrenia-like Symptoms in F1 Rats

Yawn Test

Number of yawned in a 1 hour period immediately following an acute, IP quinpirole injection (0.1 mg/kg) for F0 NS, F0 NQ, F1 FQxMQ, F1 FQxMS, and F1 FSxMQ rats is displayed in **Figure 1**. Only males were administered a yawn test, due to limits in animal availability and previous studies demonstrating that while female F0 NQ do yawn at an increased rate compared to controls, the overall effect is less robust than in males (Maple et al. 2015). As can be observed in **Figure 1**, F0 NQ rats and all three F1 groups tested demonstrated increased yawing following an acute quinpirole treatment compared to controls. As previously stated, yawning behavior is a dopamine D2 receptor mediated event (Cooper et al. 1989). Therefore, these results indicate an increase in dopamine D2 receptor sensitivity in F0 NQ animals, as well as F1 FQxMQ, F1 FQxMS, and F1 FSxMQ animals. A one-way ANOVA revealed a significant main effect of treatment group [$F(4,43)=7.68$, $p<0.001$]. Treatment group here and hence forth refers to the combination of their generation number and either neonatal treatment or parental treatment. A Newman-Keuls post hoc test indicated that while the F0 Q group and all three F1 groups demonstrated significantly increased yawning compared to controls (indicated by *, $p<0.05$), that F1 FQxMS group demonstrated significantly decreased yawning compared to the F1 FQxMQ group (indicated by #, $p<0.05$).

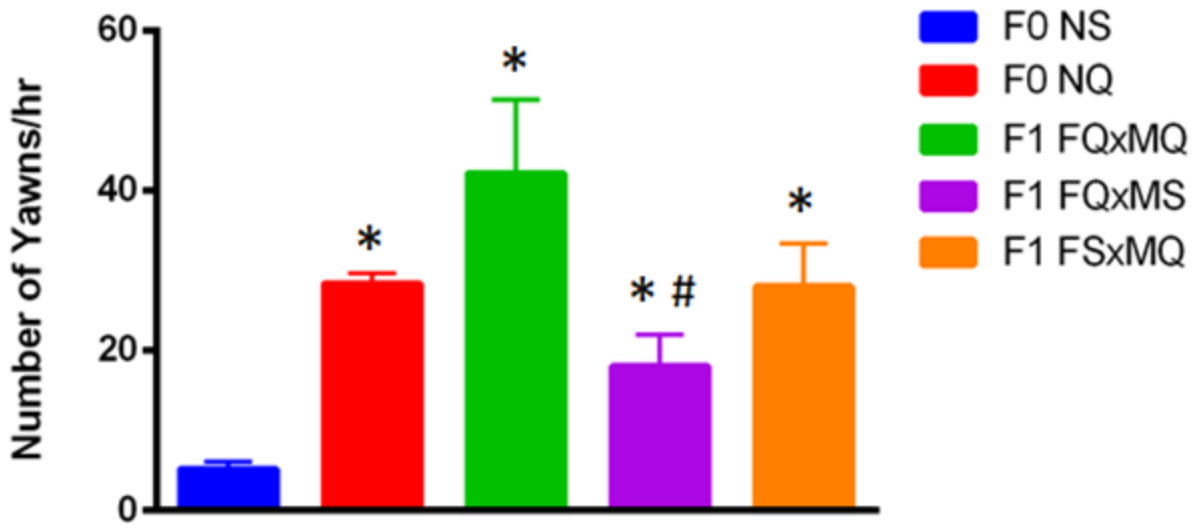

Figure 1. Number of times yawned for a 1-hour duration immediately following acute quinpirole injection (0.1 mg/kg, IP) is shown as a function of treatment group in P60-aged rats. F0 NQ, F1 FQxMQ, F1 FQxMS, and F1 FSxMQ all demonstrated significantly increased yawning compared to F0 NS rats (indicated by *, $p<0.05$). F1 FQxMS rats demonstrated significantly less yawning compared to the F1 FQxMQ group (indicated by #, $p<0.05$).

Prepulse Inhibition in Non-cross-fostered Rats

An average percent inhibition of the response to each prepulse trial decibel level was taken for each daily session of testing in male and female rats. Values of PPI obtained for each of the three daily sessions was averaged, to get overall PPI over three days of testing. Additionally, startle responses to the 120 dB trials were averaged over the 3 days of testing to determine if there were differences among the groups in basal startle response. These results are demonstrated in **Figure 2**.

Male rats. As can be seen in the **Figure 2a**, male F0 quinpirole rats, F1 FQxMQ, F1 FQxMS, and F1 FSxMQ animals all displayed decreased PPI over all 3 decibels of the prepulse compared to F0 NS controls. A 3x5 two-way ANOVA confirmed these results, with a significant main effect of treatment group [$F(4,94)=16.06$, $p<.001$] A

Newman-Keuls post hoc test revealed that all groups demonstrated significantly decreased PPI compared to controls. F0 NQ and all F1 groups did not differ from each other, indicating the level of PPI deficit did not decrease across generations. Startle response to the 120 dB trials for male rats across all three days is measured in Newtons (N) and displayed in **Figure 2b**. A one-way ANOVA revealed no difference among all treatment groups [$F(4,94)=2.2$, $p<0.071$]. Therefore, the deficit observed was the learned association between the prepulse and the startle stimuli, and not due to an effect alone on the startle response.

Female rats. In **Figure 2c**, PPI as a function of group for female rats is shown. As can be seen, female groups demonstrated less deficits overall compared to controls. However, a two-way repeated measures ANOVA revealed a significant effect of treatment group [$F(4,41)=6.09$, $p<.001$] and a significant treatment group x prepulse dB level interaction [$F(8,82)=2.72$, $p<.01$]. A Newman-Keuls post hoc test revealed that at 73 dB, F0 NQ, F1 FQxMQ, and F1 FQxMS all demonstrated significant deficits compared to F0 NS controls and F1 FSxMQ rats (indicated by *, $p<0.05$). At 76 dB, F0 NQ rats demonstrated significant a significant PPI deficit compared to all other groups. At 82 dB, there were no group differences. **Figure 2d** presents the startle responses to the 120 dB trials across 3 days (measured in N) as a function of group. Here, a one-way ANOVA did reveal a significant main effect of treatment group [$F(4,44)=4.14$, $p<.007$]. A Newman Keuls revealed that F1 FSxMQ rats demonstrated significantly increased startle response compared to all other groups (indicated by *, $p<0.05$).

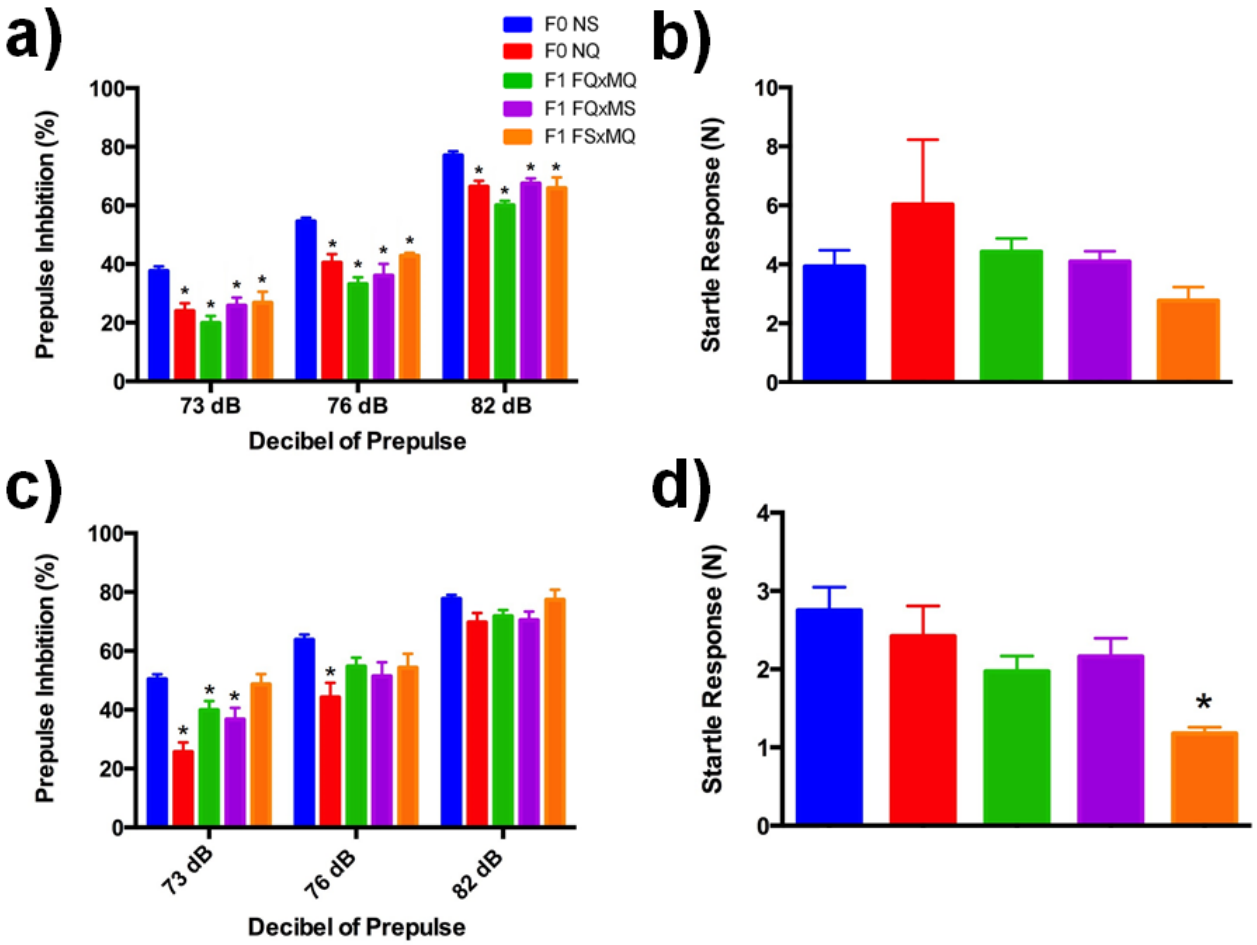

Figure 2. Average PPI and acoustic startle response in non-cross-fostered male and female rats averaged over 3 days of testing are show. An asterisk (*) denotes significant difference compared to F0 NS controls ($p<0.05$). **a)** Percent PPI for male non-cross-fostered rats is shown. All groups demonstrated significant PPI deficits compared to controls for all 3 dB levels. **b)** Acoustic startle response to 120 dB trials measured in N for male non-cross-fostered rats is shown. No groups differed compared to F0 NS rats. **c)** Percent PPI for female non-cross-fostered rats is shown. At 73 dB, F0 NQ, F1 FQxMQ, and F1 FQxMS rats differed from F0 NS controls. At 76 dB, only F0 NQ rats differed compared to F0 NS controls. No differences were found at the 82 dB level prepulse. **d)** Acoustic startle response to 120 dB trials measured in N for female non-cross-fostered rats is shown. F1 FSxMQ rats demonstrated decreased startle response compared to F0 NS controls and all other groups.

Prepulse Inhibition in Male Cross-fostered Rats

Early analysis of results of PPI in non-cross-fostered animals suggested that it was possible maternal care of F1 animals played a role in affecting PPI performance. In order to control for potential differences between NQ mothers of F1 rats and NS

mothers of F1 rats, F1 rat pups were cross-fostered to mothers of the opposite neonatal treatment group as described in the Methods and Materials section. All F0 rats were raised by their native female dam. Additionally, due to limited number of animals that were able to be cross-fostered, only males were included in this round of PPI testing.

Percent PPI over 3 days of testing was averaged for each dB level of prepulse in male rats. Startle response to the 120 dB trials was also averaged over three days. All F1 rats in this experiment were cross-fostered as described above. These results are displayed in **Figure 3**. As can be seen in **Figure 3a**, F0 NQ, F1 FQxMQ-cf, F1 FQxMS-cf, and F1 FSxMQ-cf all demonstrated decreased PPI compared to F0 NS controls and F1 FSxMS-cf rats. A 3x6 two-way ANOVA confirmed these results, with a significant effect of treatment group [$F(5,75)=17.73$, $p<0.001$] and a significant two-way interaction of treatment group and dB level [$F(10,150)=2.01$, $p<0.03$]. A Newman-Keuls post hoc test revealed that all groups demonstrated significantly decreased PPI compared to controls and F1 FSxMS-cf rats. F0 NQ, F1 FQxMQ-cf, F1 FQxMS-cf, and F1 FSxMQ-cf rats were all equivalent.

Startle response to the 120 dB trials across all three days, measured in N, is presented as a function of group and displayed in **Figure 3b**. A one-way ANOVA revealed no difference among all treatment groups [$F(4,94)=2.2$, $p<.071$].

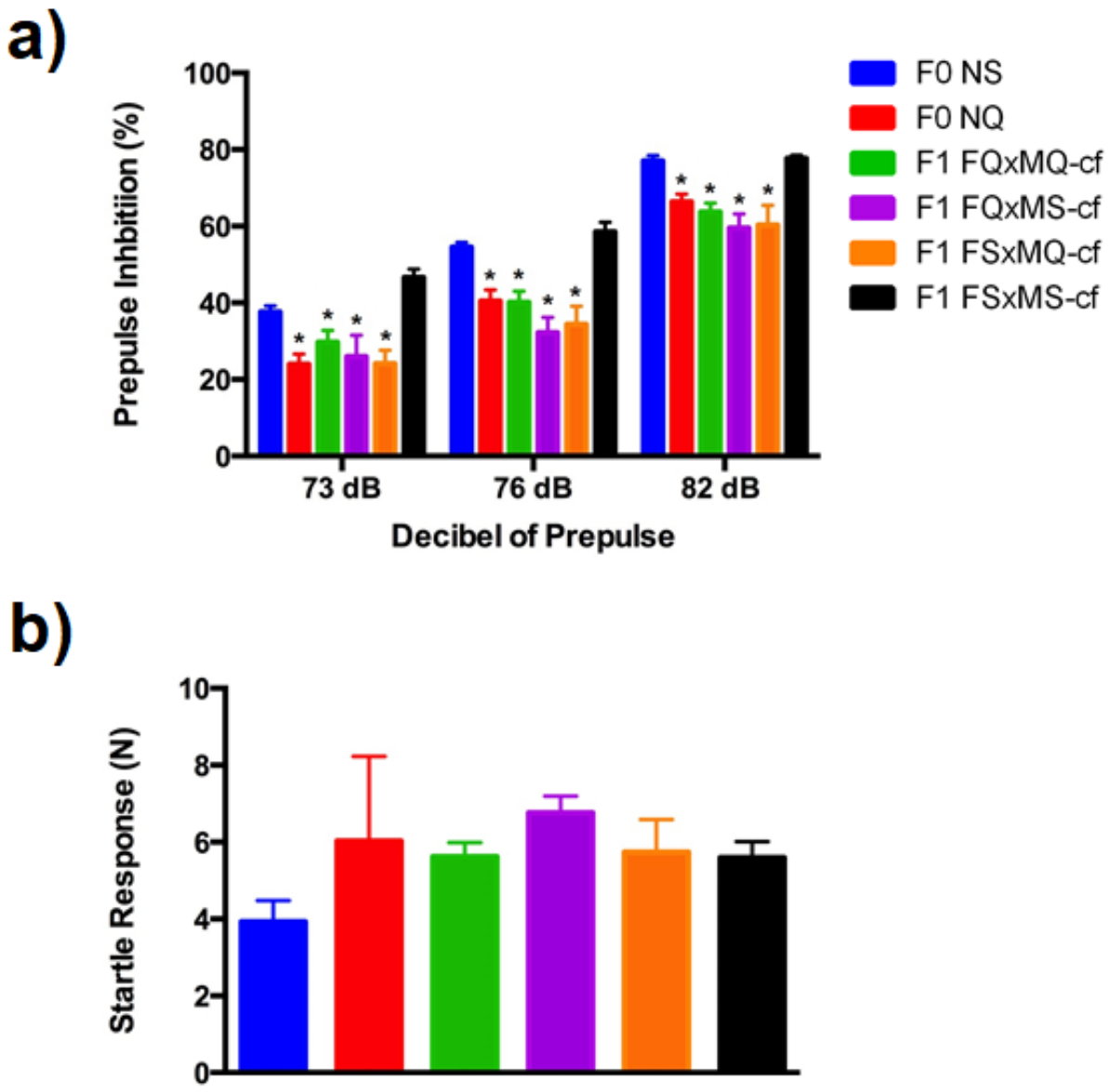

Figure 3. Average PPI and acoustic startle response in cross-fostered male rats averaged over 3 days of testing are show. An asterisk (*) denotes significant difference compared to F0 NS controls ($p<0.05$). **a**) Percent PPI for male cross-fostered rats is shown. All groups demonstrated significant PPI deficits compared to F0 NS controls and F1 FSxMS-cf rats for all 3 dB levels. **b)** Acoustic startle response to 120 dB trials measured in N for male cross-fostered rats is shown. No groups differed compared to F0 NS rats.

Transmission of Enhanced Response to Nicotine in F1 Rats

Nicotine Behavioral Sensitization

For nicotine sensitization, the unit of measure for generalized activity was distance (m) calculated as the difference in activity from Day 1 to Day 9 of nicotine treatment. A sensitization effect is described as an increase in a drug's effect on a subject after subsequent administrations of the same dose (Stewart and Badiani 1993). Therefore, the larger the sensitization effect, the larger the difference between activity from Day 1 to Day 9. Due to limitations in animal numbers, only female rats were included in this experiment. Notable from previous studies, NQ treatment produced an enhanced sensitization effect to nicotine (0.5mg/kg, IP) (Perna and Brown 2013). As can be seen in **Figure 4**, animals from all tested groups administered saline (-S) at the time of sensitization testing demonstrated no sensitization effect (Groups F0 NS-S, F0 NQ-S, F1 FQxMS-S, F1 FSxMQ-S, and F1 FQxMQ-S). F0 NS animals demonstrated behavioral sensitization to nicotine (-N) (Group F0 NS-N), and this sensitization effect was enhanced in the F0 NQ-N group as well as all 3 tested F1 groups administered nicotine at the time of sensitization testing. A two-way ANOVA confirmed these results with a significant main effect of adolescent drug treatment [$F(1,45)=72.91$, $p,0.001$]. A Newman-Keuls post hoc test revealed that F0 NQ-N rats were statistically equivalent to F1 FSxMQ-N, F1 FQxMS-N, and F1 FQxMQ-N rats; and these 4 groups were significantly greater than all other groups (indicated by **, $p<0.05$). The post hoc test also revealed that F0 SN rats demonstrated significantly increased sensitization compared to F0 NS-S rats (indicated by *, $p<0.05$), a result which validates the nicotine dose (0.5 mg/kg) chosen for the behavioral sensitization paradigm.

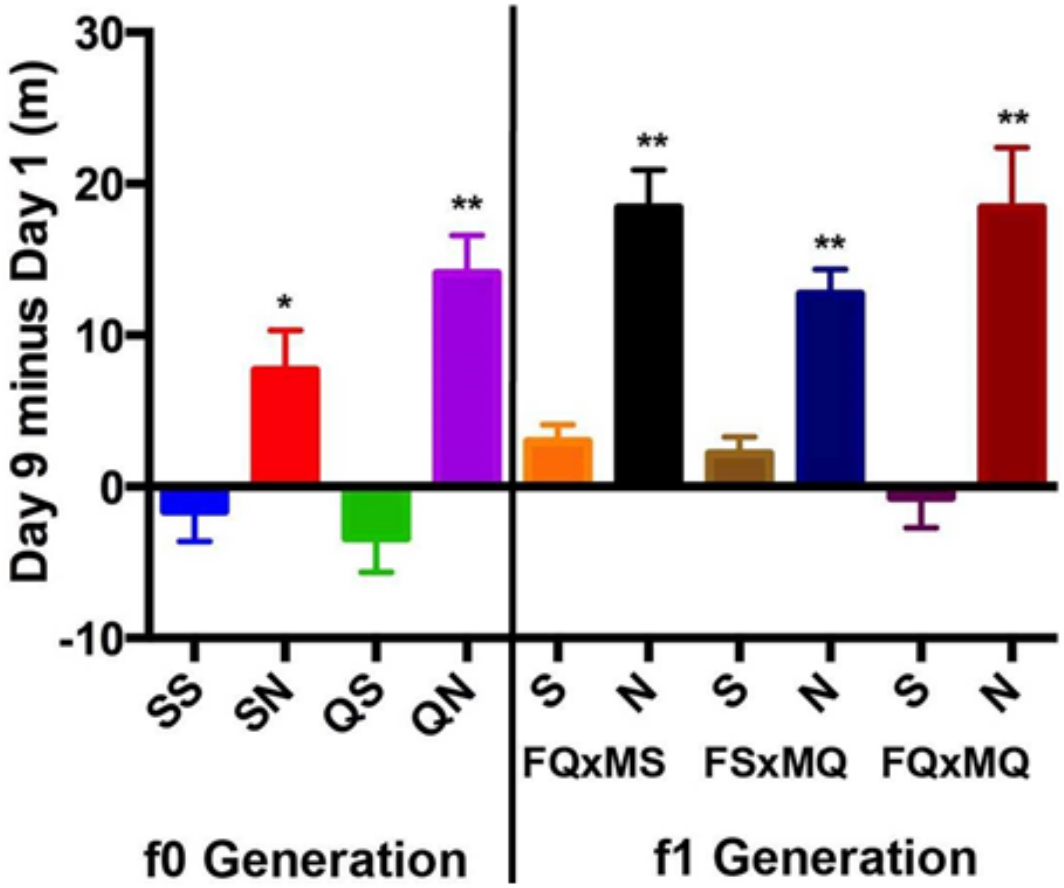

Figure 4. Behavioral sensitization is shown as a measure of general activity (meters moved in a locomotor arena) on day 9 minus distance moved on day 1 of the sensitization paradigm for each treatment group, also distinguished by whether the treatment group received nicotine (N) or saline (S) during the sensitization paradigm. The F0 SN group demonstrated increased sensitization compared to all groups which received saline at the time of testing (indicated by *, $p<0.05$). All other groups which received nicotine at the time of testing demonstrated an enhanced sensitization effect compared to the F0 SN group (indicated by **, $p<0.05$).

Nicotine Conditioned Place Preference

For CPP, the unit of measure was taken as the percent of testing time spent in the context paired with nicotine administration on the post-conditioning test minus the percent time spent in the paired context during the pre-conditioning test. Results are shown in **Figure 5.** As can be observed, rats which received saline at the time of testing (-S) did not show a preference for the paired context. All animals which received

nicotine at the time of testing (-N) did show a preference for the paired context. Compared to the control group which received nicotine (F0 NS-N), it can be observed that the F0 NQ-N, F1 FQxMQ-N, and F1 MSxFS-N groups demonstrated enhanced CPP to nicotine compared to F0 NS controls conditioned with nicotine. A one-way ANOVA revealed a significant effect of treatment group [$F(9,92)=14.32$, $p<.001$]. A Newman Keuls post hoc test confirmed that F0 NQ-N, F1 FQxMQ-N, and F1 MSxFS-N demonstrated significantly greater CPP than all other groups (indicated by **, $p<0.05$), and that F0 NQ-N and F1 FSxMQ were significantly greater than all groups conditioned using saline but demonstrated lesser CPP than the aforementioned 3 groups (indicated by *, $p<0.05$).

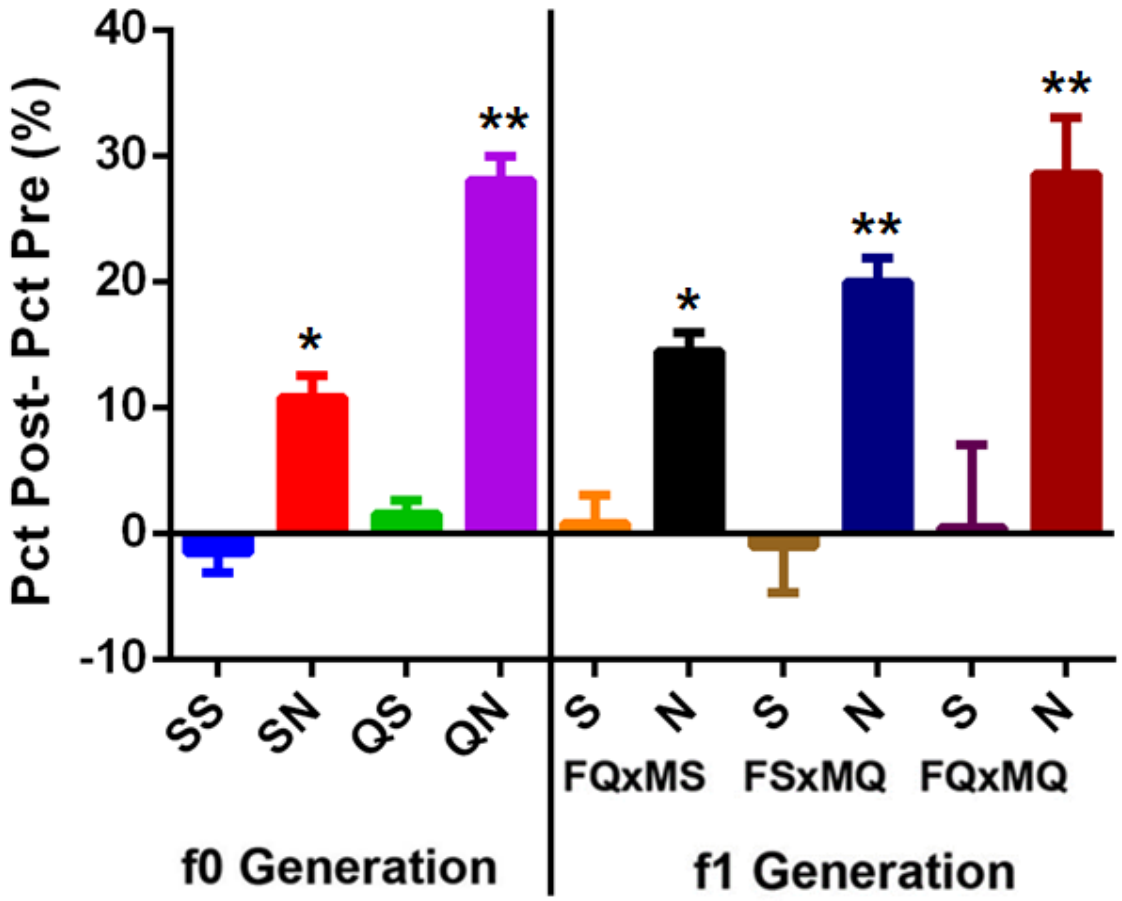

Figure 5. Nicotine CPP is shown as a measure of percent of time spent in the paired context during the post-conditioning test minus percent of time spent in the paired context during pre-conditioning as a function of treatment group, also distinguished by whether the treatment group received nicotine (N) or saline (S) during the sensitization paradigm. The F0 SN group and F1 FQxMS-N group demonstrated increased sensitization compared to all groups which received saline at the time of conditioning (indicated by *, $p<0.05$). F0 QN, F1 FSxMQ-N, and F1 FQxMQ-N all demonstrated greater CPP than all other groups (indicated by **, $p<0.05$).

BDNF Protein Expression Following Nicotine Sensitization

BDNF protein expression in the nucleus accumbens for females from the 5 treatment groups, given either saline or nicotine during the sensitization paradigm, is shown in **Figure 6.** The brain tissue analyzed was collected 24 hours following the last day of the sensitization paradigm. As can be observed in **Figure 6**, nicotine sensitization increased BDNF expression in all groups compared to saline controls. Additionally, F0 NQ, F1 FQxMS, F1 FSxMQ, and F1 FQxMQ groups all demonstrated an enhanced increase in BDNF expression following nicotine sensitization compared to F0 S animals sensitized to nicotine. A two-way ANOVA utilizing treatment group and sensitization drug treatment as factors revealed significant effects of treatment group [$F(4,49)=2.95$, $p<0.03$] and drug treatment [$F(1,49)=32.0$, $p<0.001$]. A Newman-Keuls post hoc test revealed that F0 QN, F1 FQxMS-N, F1 FSxMQ-N, and F1 FQxMQ-N groups were all significantly greater than F0 NS-S, F0 NS-N, F0 QS, F1 FQxMS-S, F1 FSxMQ-S, and F1 FQxMQ-S rats. Additionally, it was revealed that F0 NQ-S, F1 FQxMS-S, F1 FSxMQ-S, and F1 FQxMQ-S were all significantly increased compared to controls (F0 NS-S). In total, it appears that NQ treatment produces increases in BDNF protein expression which are transmitted by either parent to the F1 generation. BDNF

was also increased in animals sensitized to nicotine, and this effect was enhanced by NQ treatment, and also observed in the F1 generation.

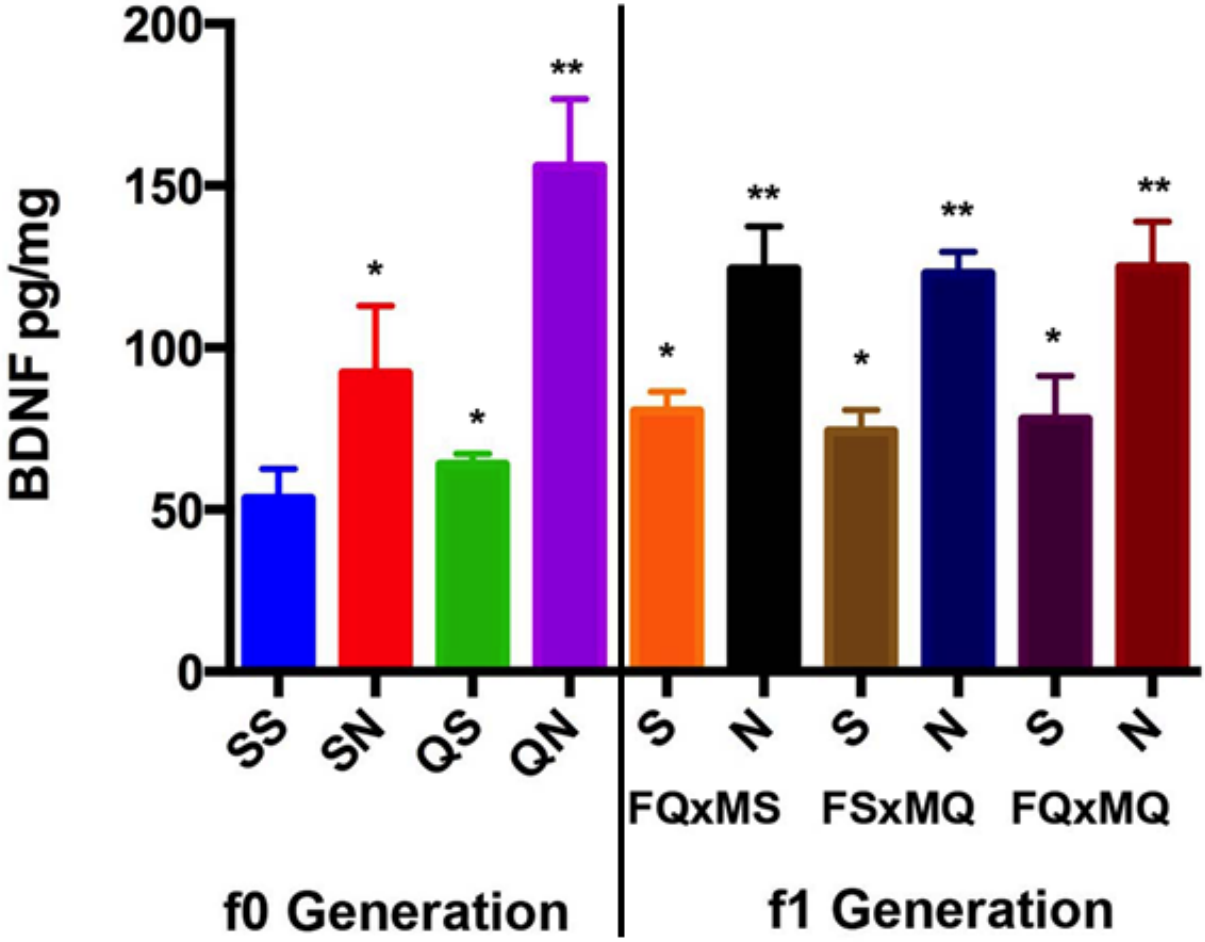

Figure 6. BDNF protein expression in the nucleus accumbens is shown as a function of treatment group, also distinguished by whether the group received nicotine (N) or saline (S) during sensitization paradigm. The F0 SN, F0 QS group, and all three F1 groups which received saline during sensitization paradigm demonstrated increased BDNF expression 24 hours after the final sensitization injection (indicated by *, $p<0.05$). F0 QN, and all three F1 groups which received nicotine during sensitization paradigm demonstrated an enhanced increase in BNDF expression compared to all other groups (indicated by **, $p<0.05$).

Suggested Mechanisms for Epigenetically Transmitted Effects

Rgs9 mRNA Expression

mRNA levels for rgs9 were assayed in the nucleus accumbens using qPCR as previously described for F0 NS, F0 NQ, and F1 FQxMQ animals. As can be seen in **Figure 7**, no difference was demonstrated between the 3 assayed groups. A one-way ANOVA confirmed this observation [F(2,17)=1.706, p=0.2112]. Due to lack of significance in primary groups in which variation in *rgs9* mRNA expression was hypothesized and supported by a previous study (Maple et al. 2007), additional treatment groups were not assayed.

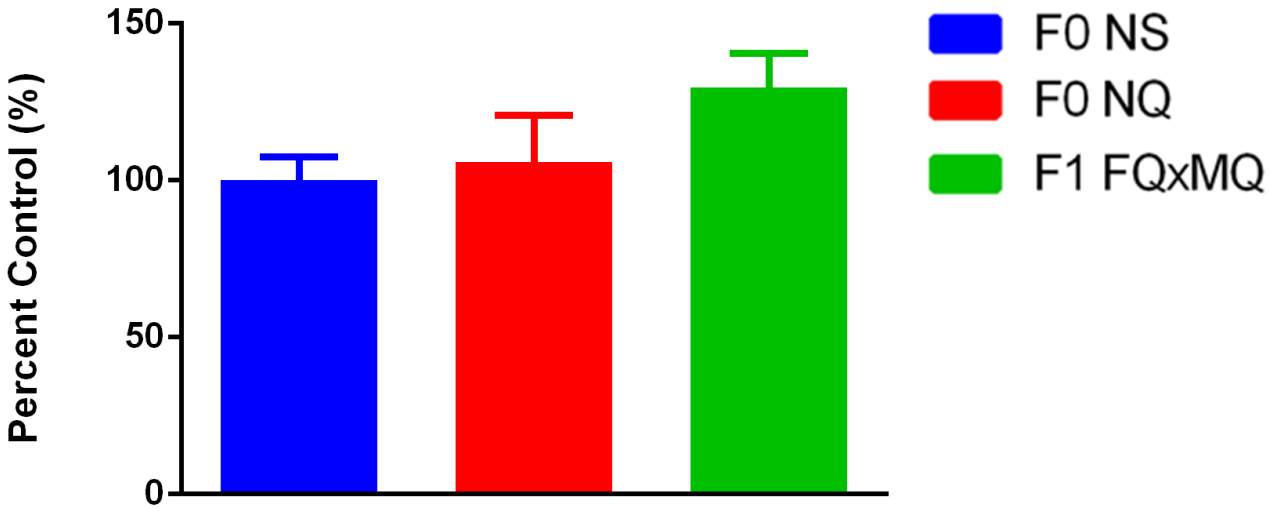

Figure 7. Percent of the control group (F0 NS) of *rgs9* mRNA expression in the nucleus accumbens is shown as a function of treatment group. Though a slight increase in mean percent control is evident in the F1 FQxMQ, no statistically significant differences were detected among the 3 assayed groups.

Rgs9 mRNA Expression Following Nicotine Treatment

mRNA levels for *rgs9* were again assayed in the nucleus accumbens using qPCR as previously described. In total, 5 groups were assayed: F0 NS-S, F0 NS-N, F1 FQxMQ-N, F1 FSxMQ-N, F1 FQxMS-N. In this analysis of *rgs9* mRNA, animals in all

treatment groups, except for a subset of F0 S animals, received 6 days of once daily nicotine injections (0.5 mg/kg, IP) starting on P54, and brain tissue was collected 24 hours following the final injection. Results in **Figure 8** are shown as a percentage difference compared to control animals (F0 S). A one-way ANOVA revealed a significant effect of treatment group [$F(4,21)=4.85$, $p<0.009$]. A Newman-Keuls post hoc test revealed that F1 FQxMQ-N demonstrated significantly increased *rgs9* mRNA.

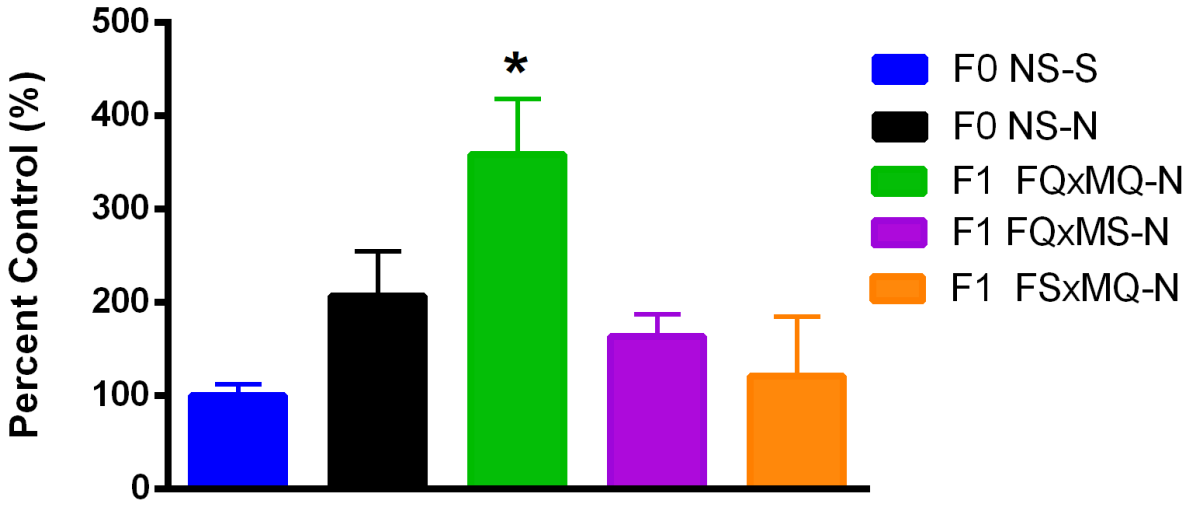

Figure 8. Percent of the control group (F0 NS-S) of rgs9 mRNA expression in the nucleus accumbens is shown as a function of treatment group. Every group except the control group received 6 days of daily nicotine injections (0.5 mg/kg, IP) prior to tissue collection. F1 FQxMQ-N demonstrated increased *rgs9* mRNA expression (indicated by *, $p<0.05$).

CREB Protein Expression

CREB protein expression in the nucleus accumbens was assayed using an ELISA kit as previously described. Three treatment groups were assayed including F0 NS, F0 NQ, and F1 FQxMQ rats. Tissue for this analysis was taken at P60. As can be seen in **Figure 9**, both F0 NQ and F1 FQxMQ groups were increased compared to F0 NS rats, which serve as controls. A one-way ANOVA confirmed this observation, finding a significant effect of treatment group on CREB expression [$F(1,26)=15.87$, $p<0.001$]. A

Newman Keuls post hoc test revealed that F0 NQ and F1 FQxMQ animals were equivalent and significantly increased compared to F0 NS animals ($p<0.05$).

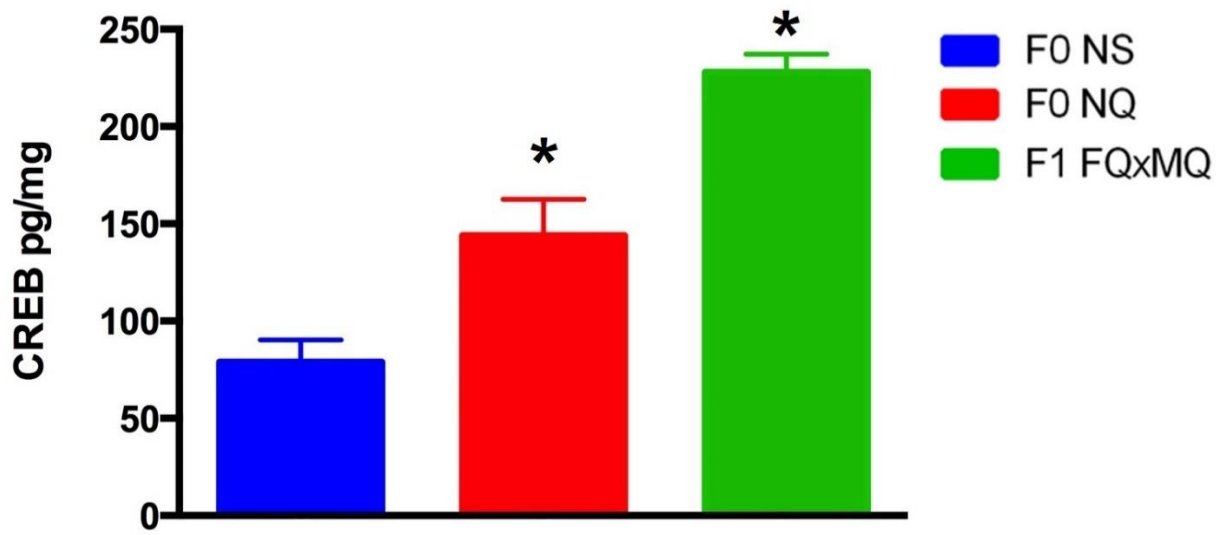

Figure 9. CREB protein expression in the nucleus accumbens is shown as a function of treatment group. F0 NQ and F1 FQxMQ groups are significantly increased compared to controls (F0 NS) (indicated by *, $p<0.05$).

CHAPTER 4

DISCUSSION

This study represents the first example of a transgenerational model of schizophrenia. Though many models utilize epigenetic mechanisms to confer schizophrenia-like symptoms in rodents (i.e. poly IC rodent model and MAM rodent model), none have examined epigenetic transmission of schizophrenia-like symptoms from parental rodents to their offspring. Several effects related to schizophrenia were demonstrated to have been transmitted to the F1 generation of the NQ rodent model. These effects can largely be explained by hypersensitivity in dopamine D2 receptor signaling, an effect which has been thoroughly studied in the NQ rodent model (Maple et al. 2007; Maple et al. 2015; Kostrzewa et al. 2016).Behavioral and neurobiological results obtained during this project suggest that dopamine D2 receptor hypersensitivity is transmitted at least one generation past the point of NQ treatment, and this effect appears to occur independent of which parental sex was neonatally treated with quinpirole.

Evidence of Epigenetic Transmission of Schizophrenia-like Symptoms

Dopamine D2 Receptor Hypersensitivity in F1 Rats

Results from the yawn test, displayed in **Figure 1**, indicate that dopamine D2 receptor sensitivity is transmitted to the F1 generation when at least one parent is treated neonatally with quinpirole. Though the yawn test relies on secondary, behavioral effects conferred by quinpirole treatment, agonist/antagonist treatment targeting the dopamine D2 receptor has indicated that yawning behavior is directly tied to receptor sensitivity (Cooper et al. 1989; Kostrzewa and Brus 1991; Collins et al. 2005).

Elevated yawning in F0 NQ rats compared to F0 NS rats replicates previous findings that NQ treatment produces increased yawing behavior following acute quinpirole treatment at P60, and thus demonstrates increases of dopamine D2 receptor sensitivity (Maple et al. 2007). Increases in yawning behavior in F1 FQxMQ, F1 FQxMS, and FSxMQ all indicate that either parent rat of F1 groups can confer increased dopamine D2 receptor sensitivity to offspring. However, it appears that female F0 NQ rats may contribute less to this effect, indicated by a lesser increase in yawning in F1 FQxMS rats. This effect is somewhat surprising, as there are many more avenues for maternal epigenetic contribution compared to relatively few paternal epigenetic contributions. Paternal contribution is largely limited to the contents of the sperm genome and epigenome. Even these contributions are diminished, as it appears that partial demethylation of paternal DNA may occurs upon zygote formation, an effect which does not appear to affect maternal genetic contribution (Oswald et al. 2000). In contrast, maternal epigenetic contribution occurs not only directly through gamete DNA, but also maternal uterus conditions, placental environment, and delivery timing (Wilhelm-Benartzi et al. 2012; Dhobale et al. 2013; Salilew-Wondim et al. 2014; Banik et al. 2017). Maternal care also plays a role in pup development, as differences in maternal care may lead to long-term epigenetic manipulations (Champagne 2008; McGowan et al. 2011; Bagot et al. 2012). Therefore, MQ animals appearing to contribute more to dopamine D2 receptor hypersensitization is a noteworthy finding. The finding that male contribution alone leads to increased dopamine D2 receptor sensitivity indicated the robustness of the dopamine-linked effects conferred by NQ treatment. Increased yawning across all F1 groups in **Figure 1** indicates the utility of the

NQ model in studying the epigenetic transmission of dopamine D2 receptor sensitivity, and its relation to schizophrenia due to the NQ model's many consistencies with the disorder.

Prepulse Inhibition Deficits in F1 Rats.

Male PPI. PPI results shown in **Figure 2a** and **Figure 3a** indicate that sensorimotor gaiting deficits are transmitted from F0 NQ rats to male F1 rats with at least one parent treated neonatally with quinpirole. Preliminary data collected during this project initially suggested there to be differences among F1 rats, which could have potentially been explained by a maternal care effect. This was due to early analysis with low sample sizes suggesting that the F1 FSxMQ did not demonstrate PPI deficits. Therefore, in order to control for a potential maternal care effect, future testing of PPI included a subset of F1 animals which were cross-fostered to a maternal rat of the opposite treatment group (**Figure 3a**). However, as rats were added to the study, mean percent PPI decreased in the F1 FSxMQ group, and that group demonstrated equivalent PPI deficits to F0 NQ and other F1 groups. Therefore, results for the non-cross-fostered and cross-fostered subsets did not differ relative to F0 NQ and F0 NS controls. Additionally, an F1 FSxMS cross-fostered group was added to the cross-fostered subset, to investigate if maternal care performed by a neonatal quinpirole-treated female would alter PPI performance in what are essentially wild-type rats. As can be observed in **Figure 3a**, the F1 FSxMS-cf group performed at similar levels to F0 NS control rats, indicating that maternal care by a rat treated neonatally with quinpirole does not alter performance on PPI in isolation. Therefore, when discussing PPI results, it is not necessary to differentiate between cross-fostered and non-cross-fostered

animals. Additionally for this reason, cross-fostering was not considered as a factor in analysis of experiments in this study except for PPI.

Male startle response. Startle response data was also collected during each PPI session and analyzed using Newtons (N) exerted by the rat on the weight-sensing plate as the measure. Startle response has been characterized as a motor reflex to a startle-inducing stimulus, and is thought to be achieved through a relatively non-complex, short pathway involving brain stem input and motor neurons (Koch and Schnitzler 1997). Altered startle response to stimuli such as the 120 dB pulse trials described above would affect PPI results, as PPI for each animal is calculated as a percent of the startle response. However, as indicated in **Figure 2b** for male non-cross-fostered rats and **Figure 3b** for cross-fostered rats, no significant difference in startle response was demonstrated across all male F0 or F1 groups.

Female PPI and startle response. PPI in female rats produced less robust deficits across treatment groups and dB level of prepulse (**Figure 2c and 2d).** Deficits in F0 NQ female rats were only present at 73 dB and 76 dB trials, and deficits in F1 rats were only present at 73 dB (F1 FQxMQ and F1 FQxMS). Additionally, female F1 FSxMQ rats demonstrated reduced startle response to 120 dB pulse trials compared to all other groups. This effect was not seen in either non-cross-fostered or cross-fostered males. These results are not entirely surprising, as sex differences have been reported in rodent PPI (Lacy et al. 2011). These sex differences could in part be explained by estrous cycle in females (Koch 1998), but the present study did not further investigate potential causes of these sex differences. It is because of the lack of robust results in

females that only males were examined in the cross-fostered subset of PPI testing. This decision was made due to limited access to cross-fostered animals.

PPI remains one of the most translationally relevant behavioral tests with respects to schizophrenia and other neurological disorders which include sensorimotor gaiting deficits as a symptom. PPI has also been tied to dopamine dysregulation. Therefore, PPI deficits in F1 rats with at least one quinpirole parent serves as further indication that dopamine dysregulation caused by NQ treatment is transgenerational. PPI deficits in humans and schizophrenia models have been shown to be alleviated by antipsychotic treatment (Kumari and Sharma 2002). Thus, PPI may serve as a screening tool for the investigation of additional treatment options as well. Further investigation of PPI performance utilizing novel intervention strategies in this transgenerational model of schizophrenia could lead to a better understanding of schizophrenia etiology as well as novel treatment options.

Evidence of Epigenetic Transmission of Enhanced Response to Nicotine

Enhanced Behavioral Sensitization and CPP to Nicotine in F1 Rats

Behavioral sensitization to nicotine. Behavioral sensitization to stimulants is an increase in response to repeated drug treatments utilizing equivalent doses. Behavioral sensitization paradigms such as the one previously described and utilized in this study are thought to model the development of addiction (Pierce and Kalivas 1997). Therefore, in a model of schizophrenia such as the NQ model, one would expect to see increases in this effect, as nicotine addiction is much more prevalent in people diagnosed with schizophrenia (Salokangas et al. 2006; Featherstone and Siegel 2015). Increased nicotine sensitization effect in F0 NQ rats has been demonstrated in multiple

studies (Perna and Brown 2013; Peterson et al. 2017). This effect appears to be in part due to an increase in accumbal BDNF, and are attenuated by antagonization of α4β2 nicotinic receptors, indicating a critical role for this receptor in mediating sensitization to nicotine (Peterson et al. 2017).

Nicotine conditioned place preference. CPP to nicotine has previously been shown to be enhanced in NQ rats (Perna et al. 2011). While robust CPP to nicotine has been shown to not present in adult rats (Clarke and Fibiger 1987), CPP to nicotine has been demonstrated in adolescent rats (Belluzzi et al. 2004). Adolescent age points are of particular importance to schizophrenia, as this is when symptoms of the disorder typically tend to manifest (Owen et al. 2016), as well as when cigarette smoking often initiates (DiFranza et al. 2000). The findings in **Figure 5** replicate past results that F0 NQ rats demonstrate an enhanced CPP effect to nicotine (0.6 mg/kg, IP) compared to F0 NS controls, and also demonstrate that this effect is transmitted epigenetically to F1 FQxMQ and F1 FSxMQ adolescent rats. These findings indicate that paternal epigenetic contribution from rats with increased dopamine D2 receptor sensitivity spawn F1 rats which are susceptible to experience enhanced associative effects to nicotine. This suggests that paternal dopamine D2 receptor sensitivity epigenetically contributes to drug abuse vulnerability in nicotine.

The present study indicates that susceptibility to enhanced nicotine sensitization present in F0 NQ animals is an effect which is transmitted to F1 rats with at least one parent treated neonatally with quinpirole (**Figure 4 and Figure 5**). While F0 NS rats did show a significant sensitization and CPP to nicotine, these effects were further enhanced by NQ treatment, as well as in the F1 generation rats with at least one

quinpirole treated parent. The only exception to this finding was that in nicotine CPP, F1 FQxMS-N rats did not demonstrate an enhanced response compared to F0 NS-N rats. Therefore, it appears that male NQ parents contribute more strongly to the transmission of enhanced behavioral response to nicotine. This finding is slightly surprising, as females are often thought to contribute more strongly to the inheritance of epigenetic effects (Guo et al. 2014; Tang et al. 2015).

Previous studies have demonstrated multiple potential mechanisms in F0 NQ rats. F0 NQ rats sensitized to nicotine demonstrate increased dopamine release in the nucleus accumbens as well as enhanced neurotrophic factor response to nicotine (Brown et al. 2012; Perna and Brown 2013). This effect in concert with increased dopamine D2 receptor sensitivity has great implications on the reward system in the brain of these rodents.

Increased Accumbal BDNF in F1 Rats Following Nicotine Sensitization

Increased BDNF protein expression following nicotine sensitization is an effect which has been previously shown in the NQ rodent model (Peterson et al. 2017). This study investigated whether this effect was transmitted to F1 rats of the quinpirole model as well, as increased BDNF protein expression in the nucleus accumbens, an area critical in mediating drug addiction, could at least in part explain the enhanced nicotine sensitization effect demonstrated by both F0 NQ rats and F1 rats with at least one parent treated neonatally with quinpirole. BDNF is a protein which has been implicated in both schizophrenia as well as addiction, and is well documented in the literature of both of these disorders (Vargas-Perez et al. 2009; Nieto et al. 2013; Chen et al. 2015; Li and Wolf 2015; Gören 2016; Bobadilla et al. 2019; Marinho et al. 2019). Results of this

study indicate that the effect of enhanced BDNF expression following nicotine sensitization previously described in F0 NQ rats is also present in F1 rats with at least one parent treated neonatally with quinpirole (**Figure 6**). Once again, it does not appear that which parental sex received NQ treatment in order to confer the potential of increased accumbal BDNF following nicotine sensitization, further indicating the strong, long-lasting effect NQ has on the epigenome. BDNF's role in maintaining plasticity at synaptic connections (Lu et al. 2015; Kowiański et al. 2018) may explain its role in mediating increased sensitization, and thus addiction, given its increased expression in the nucleus accumbens of rats which demonstrate enhanced response to nicotine.

Neurobiological Mechanisms Involved in Epigenetic Transmission to F1 Rats

Accumbal rgs9 mRNA Expression

Previous study of *rgs9* in the NQ model indicated a decrease in accumbal and striatal *rgs9* mRNA measured utilizing *in situ* hybridization (Maple et al. 2007). This effect at least in part would explain increased dopamine D2 receptor sensitivity, since as previously mentioned, rgs9 binding to dopamine D2 receptors decreases their affinity for dopamine (Cabrera-Vera et al. 2004). However, as can be seen in **Figure 7**, accumbal mRNA levels of *rgs9* via qPCR did not differ among F0 NS controls and F0 NQ or F1 FQxMQ rats. The reason for discrepancy from previous studies to this could be explained by differences in assay methods, but exact reason for this discrepancy is unclear. It is also recognized that mRNA levels may not directly correspond with protein levels, and future studies continuing the investigation of epigenetic mechanisms in the NQ model will further investigate *rgs9*.

Effect of nicotine on *rgs9* expression. *Rgs9* mRNA was also assayed via qPCR in animals which received 6 once-daily injections of nicotine (0.5mg/kg, IP). Results are shown in **Figure 8**. F1 FQxMQ-N rats were the only group demonstrated significantly altered rgs9 mRNA expression, as this group was increased. To our knowledge, this is the first time *rgs9* has been investigated in a rodent model in conjunction with nicotine administration. Increase in F1 FQxMQ-N rats following nicotine administration suggests alternations to dopamine signaling, potentially as a compensatory measure for dopamine hyperactivity in the nucleus accumbens following nicotine administration, as dopamine increases in the nucleus accumbens have been reported in F0 NQ rats following nicotine administration (Perna and Brown 2013). Given F1 FQxMQ rats' consistencies with F0 NQ rats, it would make sense that this effect may also be present in F1 generation animals. However, the exact mechanism for the increase in rgs9 expression following nicotine administration is unclear, and why this effect is not seen in additional groups is also unknown.

Accumbal CREB Expression

Though CREB is expressed throughout the brain, alterations in its expression in the nucleus accumbens have been linked to symptoms consistent with schizophrenia including PPI deficits (Culm et al. 2004) and blunted emotional response to aversive stimuli (Carlezon et al. 2005). In addition, increases in CREB activity have been linked to increased dopamine D2 receptor activation (Yan et al. 1999). Here, we demonstrate that accumbal CREB is increased in both F0 NQ rats as well as F1 FQxMQ rats compared to control animals (**Figure 9**). However, there are no clinical data showing changes of CREB in the nucleus accumbens in the post-mortem brain tissue of

individuals diagnosed with schizophrenia. The roles of CREB in the pathology of schizophrenia have been discussed in a recent paper by Wang and colleagues. Studies have shown that dopamine, antipsychotic drugs, growth factors, and other schizophrenia-related genes could activate CREB and its downstream target BDNF via different pathways (Wang et al. 2018). Alterations of CREB/BDNF signaling have been found in schizophrenia, but the precise characterization of these mechanisms are not currently clear. Preclinically, it has been shown that chronic quinpirole to adult rats produces an improvement in PPI and decreases of CREB in the nucleus accumbens (Culm et al. 2004). This may appear to contradict our results. However, chronic quinpirole treatment to adult rats is known to produce a decrease of dopamine D2 receptor density, and result in downregulation of dopamine D2 receptors (Subramaniam et al. 1992). Since NQ treatment results in an increase of dopamine D2 receptor sensitivity, but not change in receptor number, it is possible that CREB is positively correlated with changes in dopamine D2 receptor signaling, with increases in D2 signaling resulting in an increase of accumbal CREB, and a decrease in D2 signaling associated with decreased accumbal CREB. While this conclusion is speculative, the data appear to support this explanation as a possibility. Although this result does not necessarily mechanistically explain dopamine D2 receptor hypersensitivity seen in F0 or F1 rats of the NQ model, it does at least in part explain downstream behavioral effects such as sensorimotor gaiting deficits seen in previous studies in F0 rats (Maple et al. 2015) and in this study in F0 and F1 rats.

Limitations

Proving transgenerational epigenetic transmission poses many challenges. For example, it is possible that quinpirole exerts off-target environmental impact such as cytotoxicity on germline cells that results in altered phenotype in the F1 generation, which is not the same mechanism of increased dopamine D2 receptor sensitivity demonstrated in F0 NQ rats. In order to rule out environmental impact of quinpirole or other environmental stimuli on germline and other supporting cells in the neonatally treated breeder rats, at least one generation succeeding F1 animals must be studied. Investigation of an F2 generation and succeeding generations is especially important when lacking evidence of specific epigenetic mechanisms demonstrated to be present in germline cells (Horsthemke 2018).

Though this study details a case for the existence of epigenetic mechanisms of transmission of schizophrenia-like effects in the NQ rodent model, a thorough investigation into those mechanisms was not performed. Factors contributing to this deficiency include both resources and a lack of a robust understanding of mechanisms contributing to dopamine D2 receptor hypersensitivity in the F1 generation of the NQ model. The behavioral establishment of this model was the priority of this particular study, and a complete investigation into the mechanisms underlying these effects is an immense task. Regardless, investigation into specific epigenetic mechanisms in germline cells, and behavioral testing of an F2 generation of animals could rule out mechanisms of non-epigenetic transmission of the behavioral and neurobiological phenotype demonstrated in this study.

Additionally, due to restrictions of time and rat availability, sample sizes were not large enough in many of the performed experiments to be able to analyze potential sex differences with sufficient statistical power. Sex differences have previously been detailed in the NQ rodent model (Perna and Brown 2013; Maple et al. 2015; Peterson et al. 2017) and human schizophrenia (Mendrek and Mancini-Marïe 2016; Gonçalves et al. 2019). Therefore, analysis of sex differences with regards to epigenetic transmission of schizophrenia-like phenotype in the NQ model could lead to improvements in current schizophrenia diagnosis and treatment. Future continuation of this line of research will serve to allow these analyses.

Conclusions

Overall, this study provides evidence of epigenetic transmission of schizophrenia-like effects in the NQ rodent model of schizophrenia. These findings provide evidence of epigenetic transmission of dopamine D2 receptor hypersensitivity, which builds upon the relatively small knowledge of the epigenetic impact of schizophrenia-like conditions. For instance, data presented in **Figure 1** indicates that dopamine D2 receptor hypersensitivity in a parent is transmitted to offspring, even if only one parent is afflicted. Though this effect likely presents more strongly in rodents than it would in the human population due to the much smaller level of variability and other confounding factors exhibited in lab rodents, its examination in isolation demonstrates that alterations in dopamine signaling have potential to be robustly transmitted epigenetically. Transmission of sensorimotor gaiting deficits to F1 rats with at least one F0 NQ parent demonstrates that neurobiological alterations caused by drug

treatment, or potentially neurological environment in neurological disorders, can be epigenetically transmitted to mediate behavioral phenotype as well.

This study also provides evidence that drug abuse vulnerability is caused by dopamine D2 receptor hypersensitivity, and this effect is epigenetically transmitted to F1 rats. Increased sensitization and CPP to nicotine indicate NQ rats model drug abuse vulnerability, an effect which could partially be attributed to increases in neurotrophic factors such as BDNF following nicotine administration. Further studies into mechanisms of epigenetic transmission which allow for enhanced response to nicotine to manifest across multiple generations of the NQ model would help to better understand risk factors of drug abuse, allowing for preventative or earlier intervention in individuals vulnerable to drug abuse development. Given the high level of abuse of nicotine among individuals diagnosed with schizophrenia, understanding the epigenetic link between dopamine D2 receptor hypersensitivity and nicotine abuse would also help to fill knowledge-gaps about the etiology of schizophrenia, other behavioral disorders that present with psychosis, and their high rate of comorbidity with nicotine abuse. Due to the high rate of treatment resistance in schizophrenia (Taylor and Duncan-McConnell 2000), and many individuals likely relying on nicotine to relieve negative symptoms associated with schizophrenia (LeDuc and Mittleman 1995), better understand of the largely unknown epigenetic components of the link between schizophrenia and nicotine abuse could lead to novel treatment options.

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Peterson, D. J., Gill, W. D., Dose, J. M., Hoover, D.B., Pauly, J. R., Cummins, E. D., Burgess, K. C., & Brown, R. W. (2017). The effects of nicotine in the neonatal quinpirole rodent model of psychosis: Neural plasticity mechanisms and nicotinic receptor changes. Behavioural Brain Research, 325, 17–24.

Honors and Awards:

ETSU Graduate Student Research Grant, East Tennessee State University, 2017,2018

ETSU Biomedical Sciences Student Association Vice President, East Tennessee State University 2016-2019