ASSESSING MECHANISMS DRIVING INCREASED NICOTINE USE IN SCHIZOPHRENIA USING THE MAM RODENT MODEL

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Rates of smoking are 2-3 times higher among individuals with schizophrenia relative to the general population, posing a significant exacerbation of health and socioeconomic disparities. While the cause of this elevation is unknown, two prevalent hypotheses are that nicotine, the primary reinforcing component in cigarettes, is particularly rewarding to individuals with schizophrenia, or that these smokers smoke in an attempt to "self-medicate" their symptoms. The present study utilized the methylazoxymethanol (MAM) neurodevelopmental rodent model of schizophrenia to evaluate these hypotheses. In order to explore the "reinforcement hypothesis", MAM and control (CTL) animals were allowed to self-administer nicotine across a range of doses and responding and intake of nicotine were compared. The "self-medication" hypothesis was evaluated by examining the effects of acute and chronic nicotine on several established behavioral paradigms known to be disrupted in the MAM model. Additionally, the effects of acute and chronic nicotine on neurophysiological dysfunction, including elevated VTA dopamine population activity and elevated ventral hippocampal (vHipp) activity, were observed. Our findings demonstrated that self-administration of nicotine, alone or in combination with another reinforcer, was not increased in MAM rats, suggesting that schizophrenia pathophysiology modeled by MAM does not confer increased nicotine reinforcement. Conversely, we observed nicotine-induced improvements in prepulse inhibition of startle and novel object recognition among MAM rats, as well as a

normalization of elevated VTA dopamine and vHipp neuronal activity in these animals. Together, these findings lend greater support for a "self-medication" hypothesis behind increased smoking in schizophrenia and illustrate the potential utility of nicotinic modulation in future pharmacotherapies for certain schizophrenia symptoms.

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PREFACE

The work presented in Chapter 2 is based on a previously published manuscript: Weeks JJ, Rupprecht LE, Grace AA, Donny EC, & Sved AF. Nicotine Self-administration is Not Increased in the Methylazoxymethanol Acetate Rodent Model of Schizophrenia, Nicotine & Tobacco Research, 2020, Volume 22, Issue 2, Pages 204-212, by permission of the Society for Research on Nicotine & Tobacco.

1.0 INTRODUCTION

1.1 CIGARETTE SMOKING IN SCHIZOPHRENIA: AN OVERVIEW

Cigarette smoking remains the largest preventable cause of death in the United States, contributing to multiple serious diseases and impacting overall health of smokers (U.S. Department of Health and Human Services 2014). Overall smoking rates have decreased steadily over time as public awareness of these health risks has grown. Currently, an estimated 13-15% of U.S. adults are smokers, though this figure encompasses a tobacco use and disease burden disproportionately affecting vulnerable populations (Drope et al. 2018). In particular, higher smoking rates are reported among those with serious mental illnesses (SMI), with the highest prevalence among schizophrenia (SCZ) patients. SCZ is a chronic and severe mental disorder that affects roughly 1% of the population. It disrupts an individual's ability to process and perceive reality clearly and is characterized by a variety of symptoms, including delusions, auditory and visual hallucinations, disorganized speech and behavior, anhedonia, and cognitive impairments (McCutcheon et al. 2019). A study by Dickerson and colleagues (Dickerson et al. 2018) spanning 1999-2016 found that, overall, 62% of SCZ patients were smokers, compared with 37% of those with bipolar disorder and 17% of controls. Moreover, roughly half of all deaths in individuals with SMI are attributed to tobacco-related illness, making smoking cessation in this population an urgent priority (Callaghan et al. 2014).

In addition to being more likely to smoke overall, individuals with SCZ display a distinctly heavy pattern of smoking (de Leon and Diaz 2005). Smokers with SCZ smoke more cigarettes per day than controls, are more likely to be nicotine (NIC) dependent, and tend to smoke more

intensely (Dickerson et al. 2018; Stewart et al. 2013). A study of smoking topography found that smokers with SCZ take more puffs per cigarette, with larger puff volumes, shorter inter-puff intervals, and greater levels of expired carbon monoxide than controls without SMI (Tidey et al. 2005). This intense pattern of smoking contributes to high rates of smoking-related illness, exacerbating the massive health disparity faced by this population. For example, smokers with SCZ are significantly more likely than controls to be diagnosed with respiratory diseases, tobacco-related cancers, and cardiovascular diseases (Krieger et al. 2019; Tran et al. 2009) Individuals with SCZ face an overall estimated reduction in life expectancy of 13-15 years relative to the general population (Hjorthøj et al. 2017). Smoking furthers this discrepancy within the patient population. An 11-year prospective study of SCZ patients by Dickerson and colleagues (Dickerson et al. 2014) found that those who smoked were 5 times more likely to die of "natural causes" (largely encompassing cardiovascular ailments) than nonsmoking patients. Despite these clear health consequences, success in smoking cessation in this population has been limited by a number of factors. Smokers with SCZ are more likely to experience greater barriers to cessation, including higher levels of dependence, prosmoking social norms, financial stress, unemployment, and a lack of access to cessation support or nicotine replacement therapies (Lum et al. 2018). Unfortunately, this discrepancy is also perpetuated in both inpatient and outpatient treatment. Tobacco use is often a low priority issue in primary care settings for SCZ patients due to the complex competing demands of their condition (Cerimele et al. 2014). Additionally, psychiatrists, who are main consistent healthcare contact for many patients, are less likely than other providers to encourage smoking cessation (Hall and Prochaska 2009). This is despite the fact that smokers with SCZ generally report a high level of motivation to quit, even among patients with greatest symptom severity (Anzai et al. 2015). In inpatient psychiatric care settings, smoking bans remain a

contentious issue in ethics and patient autonomy, since many patients cannot leave (Woodward and Richmond 2019). Smoking places a major financial burden on SCZ patients as well, many of whom live on fixed, limited incomes. A study of SCZ smokers on public assistance found that an average 30% of their monthly income was spent on cigarettes, severely limiting access to other resources that may improve quality of life (Steinberg et al. 2004). There is a growing awareness of the urgent need for smoking cessation among patients with SCZ, but the unique barriers faced by this population require distinct approaches to reaching this goal.

1.2 A REINFORCEMENT-BASED HYPOTHESIS OF INCREASED SMOKING IN SCHIZOPHRENIA

While the high rate of smoking among individuals with SCZ is well-documented, the underlying etiology remains unclear. This discrepancy cannot be attributed purely to environmental and psychosocial factors, as it persists even when controlling for factors such as socioeconomic status, institutionalization, employment, and education level (Hughes et al. 1986). A commonly suggested hypothesis is that SCZ confers neurobiological, reward-related risk factors that contribute to tobacco use beyond the psychosocial factors that limit cessation. Indeed, rates of co-occurring substance use disorders (SUDs) are high among those with SCZ, with overall reported SUDs occurring at more than twice the rate of those in the general population. Addiction to nicotine (NIC), the primary psychoactive and reinforcing component of tobacco, is widely accepted as the main driver of tobacco smoking and represents the most prevalent SUD among patients (Volkow 2009). A common proposal suggests that the hippocampal-prefrontal dysregulation of dopamine (DA) release in the nucleus accumbens (NAcc) observed in SCZ may

contribute to positive symptoms of the disease as well as a heightened addiction vulnerability (Chambers et al. 2001). This vulnerability is largely associated with an impaired sensitivity to reward observed in SCZ patients. Anhedonia, a reduction in capacity for pleasant and rewarding emotion, is a hallmark symptom of SCZ that is often resistant to treatment (Horan et al. 2006). Anhedonia in this population largely manifests as a reduction in anticipatory pleasure; studies suggest that SCZ patients do not differ from controls in self-reports of current pleasant mood, but tend to underestimate expected pleasure from future events and experienced pleasure from past events (Strauss 2013). This suggests that anhedonia in this population is more related to how patients conceptualize, anticipate, and learn about reward, rather than how reward is experienced in the present.

Many studies suggest that reward-related dysfunction is related to increased smoking among SCZ patients. In a 2012 study by AhnAllen and colleagues (2012), SCZ and control smokers were administered a signal detection task to assess reward-based learning. While SCZ smokers did not differ from controls in overall patterns of reward-based learning, decreased performance was associated with increased NIC dependence and increased anhedonia specifically among patients. This suggests that reward dysfunction may drive NIC intake, particularly in those patients with the highest levels of dependence. The unique reinforcing effects of NIC may explain why the use of tobacco is so prevalent. Like other drugs of abuse, NIC directly stimulates phasic DA release in striatum, which is associated with its rewarding effects (Rice and Cragg 2004). A wealth of both clinical and preclinical data have established that, in addition to its primary reinforcing effects, NIC increases responsivity to non-drug rewards, an effect known as reinforcement enhancement (Perkins et al. 2017; Rupprecht et al. 2015). Thus, NIC may mitigate

an underlying reduction in hedonic capacity and reward-based learning in smokers with SCZ by increasing responsivity to reward in general.

In addition to studying the effect of smoking on reward-based learning, clinical studies related to this phenomenon have focused on comparing the rewarding effects of smoking relative to other rewards in SCZ smokers versus control smokers. A study by Spring and colleagues (2003) sought to assess perceived smoking reward in heavy smokers with SCZ, depression (a disorder also characterized by anhedonia), and nonpsychiatric controls. Subjects were asked to rate their perception of relative benefits versus drawbacks of smoking to create a pro/con "balance sheet" while controlling for dependence as measured by Fagerstrom Test for Nicotine Dependence (FTND). Despite acknowledging risks and drawbacks of smoking, both groups of patients were more likely to rate the overall effects of smoking as positive, while nonpsychiatric controls tended to find nearly equal pros and cons. Subjects also completed a forced-choice task where they made hypothetical selections between smoking and alternative rewards such as receiving a gift or eating a favorite candy. Psychiatric subjects were twice as likely than controls to select smoking over alternative rewards, and smokers with SCZ did not differ from those with depression (Spring et al. 2003). These results suggest that heavy smokers with depression or SCZ, conditions marked by reward processing dysfunction, may find smoking more rewarding than controls.

More targeted assessments of motivation to smoke provide greater insight into the reinforcement hypothesis as well. A behavioral economic assessment of smokers with SCZ by MacKillop & Tidey (2011) aimed to characterize cigarette demand and delayed reward discounting, a behavioral economic measure of impulsivity. In prior studies of delayed reward discounting, individuals with SCZ tended to discount delayed monetary rewards more steeply than nonpsychiatric controls regardless of smoking status, suggesting a higher level of impulsivity and

a preference for immediate over long-term rewards (Heerey et al. 2007). This preference is consistent with established impairments in evaluation of future or past rewarding experiences in SCZ (Strauss 2013). Interestingly, MacKillop and Tidey (2011) found that SCZ smokers demonstrated less impulsive delay discounting than control smokers, suggesting that impulsive reward preferences may not be a variable motivating particularly high smoking in this population. Subjects' demand for cigarettes was also assessed using a hypothetical cigarette purchase task (CPT), where subjects were asked to report how many cigarettes they would smoke at a given price if they were available currently (i.e. they could not be stockpiled and smoked at a later time). Smokers with SCZ demonstrated significantly greater intensity of demand than control smokers. While they were sensitive to changes in price, SCZ subjects reported higher levels of expenditure (i.e. greater hypothetical consumption of cigarettes) in the CPT at the five lowest price points. In general, this finding is consistent with established evidence that smokers with SCZ smoke more cigarettes per day and spend a greater proportion of their income on cigarettes than control smokers, and does suggest differences in the valuation of cigarettes between these populations (Dickerson et al. 2018; Steinberg et al. 2004).

Behavioral demonstration of the reinforcing value of cigarettes as a piece of evidence for a "reinforcement hypothesis" behind increased smoking is challenging. A second popular theory explaining high rates of tobacco use in SCZ is the "self-medication hypothesis", which suggests that smoking serves as a means of normalizing or alleviating underlying pathology of the disease (Leonard et al. 2007). It is difficult to fully disentangle a greater subjective rating of smoking in SCZ patients from a broadly defined self-medication hypothesis, as self-medication could encompass feeling "better", which is reinforcing in itself, without conscious association to symptoms. The high incidence of smoking initiation prior to the onset of active psychosis could

suggest a mechanism driving increased smoking vulnerability that precedes the full transition to SCZ. In general, the majority of smokers begin the habit in adolescence, a period prior to the emergence of most SCZ symptoms. For example, a study of 237 smokers with SCZ found that 86% of subjects began smoking more than a year prior to disease onset, with a mean age of 18.7 years at smoking initiation and 24.1 years at onset of psychosis (Beratis et al. 2001). A longitudinal study of males screened in late adolescence and early adulthood found that individuals who smoked 10 or more cigarettes per day as teens were 2.28 times more likely to be later diagnosed with SCZ, further suggesting a higher rate of smoking prior to the emergence of symptoms (Weiser et al. 2004). However, some evidence suggests that smoking initiation may overlap with the prodromal phase of SCZ, a period preceding psychosis by weeks to years. During the prodromal phase, individuals typically first experience symptoms such as depression, anxiety, and social withdrawal, followed by brief, infrequent, and mild thought disturbances and predelusions prior to psychosis (Larson et al. 2010). Interestingly, some evidence suggests that individuals with SCZ start smoking closer to symptom onset than smokers diagnosed with nonpsychotic mental illness, which could imply an overlap in smoking initiation and prodromal syndrome (Riala et al. 2005). A study of NIC use in young people designated as ultrahigh-risk (UHR) for psychosis, a formal classification of prodromal syndromes, found that 46% of UHR subjects were smokers, compared to 22% of age-matched controls. Additionally, UHR subjects reported significantly more frequent use of NIC than controls (Gupta and Mittal 2014). While the majority of evidence indicates that individuals with SCZ begin smoking prior to the full emergence of psychosis and are more likely to smoke than controls, it is unclear if this is driven by prodromal symptoms or is the result of an intrinsic vulnerability to both SCZ and NIC dependence.

1.3 SMOKING AS SELF-MEDICATION IN SCHIZOPHRENIA

The "self-medication hypothesis" theorizes that individuals with SCZ smoke at an increased rate in order to normalize symptoms of the disease or alleviate side effects of antipsychotic drugs (APDs) (Leonard et al. 2002). As previously mentioned, this may not be mutually exclusive from a "reinforcement hypothesis", as some of the features of SCZ thought to motivate smoking, such as reward dysfunction and anhedonia, could also be potentially alleviated by NIC intake. SCZ is characterized by several general domains of symptoms. Positive symptoms encompass elements of psychosis, such as hallucinations and delusions, while negative symptoms refer to a reduction in volition leading to a lack of motivation, reduction in speech, and social withdrawal. Changes in neurocognition are also prominent, such as difficulties with memory, attention, and executive function, as well as previously described affective symptoms, such as anhedonia (van Os and Kapur 2009). While the neurobiology of SCZ is not fully understood, there is a great deal of established knowledge about circuit pathologies associated with these changes, many of which reflect a general alteration in the balance of excitation and inhibition throughout regions of the brain (Ferguson and Gao 2018; Gao and Penzes 2015)

Positive symptoms are largely attributed to increased activity of ventral tegmental area (VTA) DA neurons, the firing patterns of which affect DA release in numerous efferent targets throughout the brain. DA signaling from VTA to NAcc is critical for attributing appropriate salience to relevant cues and stimuli based on sensory input and prior expectations. Briefly, hyperresponsivity of the mesolimbic DA system produces an increase in background DA activity and a decreased "signal to noise ratio" in encoding relevant stimuli, contributing to hallucinations and delusions (Sterzer et al. 2018; Kapur 2003). Imaging studies show increased DA synthesis as well as increased synaptic DA in the mesolimbic pathway during psychosis (Howes et al. 2012).

This disruption also contributes to impairments in sensory gating widely observed in SCZ patients and first-degree relatives. Sensory gating refers to a neurological process of filtering out unnecessary or redundant stimuli from all perceived environmental stimuli and is reflected in measures such as inhibition of the auditory startle response by presentation of a brief prepulse stimulus (Swerdlow et al. 2016). Additionally, dysfunction in DA signaling of relevant stimuli also reduces their motivational salience, producing changes in reward-associated learning. Concurrently, changes in glutamate and DA signaling as a result of receptor feedback reduce prefrontal cortical DA release and contribute to deficits in working memory, attention, and executive function (Slifstein et al. 2015). Together, these alterations are hypothesized as a mechanism underlying certain aspects of both negative and cognitive symptoms of the disease, as this frontal-striatal connection is necessary for meaningfully translating reward-associated striatal DA signals to guide motivated behaviors (McCutcheon et al. 2020; Howes et al. 2012). Negative symptoms affecting reward have an overlapping impact on cognitive functioning, as motivation, effort allocation, and value representation are active processes in many cognitive demands (Robison et al. 2020). A number of studies have indicated that severity of negative symptoms, cognitive impairments, and psychosocial functioning are strong predictors of overall patient outcome in SCZ, which highlights the need to understand and effectively manage these symptoms (Milev et al. 2005; McGurk et al. 2000). While antipsychotic drugs (APDs) have been available for decades, efforts to develop successful pharmacotherapies to mitigate cognitive dysfunction in SCZ have been largely unsuccessful (Goff et al. 2011).

Much of the research behind the self-medication hypothesis proposes that the action of NIC on $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) throughout the brain provides a pro-cognitive effect. Associations of nicotinic function with cognitive effects are behaviorally supported by wide

evidence of NIC administration enhancing cognitive performance in both smoking and nonsmoking controls (Heishman et al. 2010). Interest in the relationship of α7 nAChRs and SCZ grew from several converging lines of neurobiological, behavioral, and genetic findings. Polymorphisms and partial duplication of CHRNA7, the gene for the α7 nAChR subunit, have been associated with SCZ diagnosis and disease-related deficits, including impaired prepulse inhibition of startle (PPI) and visual information processing, as well as smoking prevalence (Bakanidze et al. 2013; Stephens et al. 2012; Leonard et al. 2007) Critically, a significant correlation between neuropsychological performance (measured by the Wisconsin Card Sorting Task) and PPI deficits, which are largely considered two distinct domains, has been demonstrated specifically in SCZ smokers but not nonsmokers (Rabin et al. 2009). This may suggest a unique mechanistic link between NIC use and both cognitive and sensorimotor gating deficits in SCZ.

A test of the hypothesis that individuals with SCZ smoke as a means of self-medication can be approached from multiple directions. An intuitive question is whether symptoms are more or less severe in patients who smoke, as a potential indication of NIC intake reducing symptom severity. Alternatively, this relationship could be examined from the reverse: are individuals with more severe symptoms smoking more? Many studies suggest an association between smoking status, greater severity of negative and cognitive symptoms, and poorer outcomes (Iasevoli et al. 2013; Reed et al. 2016). A 2007 study of SCZ outpatients examining the relationship between PANSS (Positive and Negative Syndrome Score) scores and level of NIC dependence found significant associations between the two. Specifically, high total PANSS scores, indicating more severe symptoms, were significantly more likely in highly dependent and nonsmoking patients than in mildly dependent smokers, but that scores of negative symptoms, which are often associated with the self-medication hypothesis, were not associated with NIC dependence. Highly

dependent smokers also had the greatest proportion of lifetime hospital admissions (Aguilar et al. 2005). A community mental health study of individuals with SCZ found no association between smoking severity (i.e. cigarettes smoked per week) and scores of positive and negative psychiatric symptoms, though this study did not include any assessment of cognitive function (Dixon et al. 2007). A larger study of multiple diagnostic groups found that SCZ patients were more likely than any other group to smoke heavily (1+ pack per day), and that greater symptom severity was associated with heavy smoking (Vanable et al. 2003). However, in UHR young adults, NIC use was associated with greater performance in several cognitive domains, suggesting that smoking could improve some initial symptoms, though it is unclear whether this relationship would persist after psychosis onset (Gupta and Mittal 2014). It is challenging to draw conclusions on the self-medication hypothesis by examining the relationship between symptom severity and smoking frequency because of differing possible interpretations of the same finding. For example, higher levels of smoking in individuals with greater symptom severity could suggest that severe symptoms are driving those patients to smoke more. Conversely, the presence of severe symptoms in this population would also fail to support self-medication if the heavy smoking is posited to provide some relief of these symptoms.

Antipsychotic drug (APD) treatment may also impact rates of smoking among individuals with SCZ. While a detailed exploration of APD subtypes, mechanisms of action, and relationships with NIC use and behavior is beyond the scope of this document, a summary of key trends is warranted. Broadly, APDs fall into two classes. Typical, or first-generation, APDs exert their action primarily through antagonism of D2 DA receptors, while atyptical (second-generation) APDs have greater heterogeneity in their mechanisms of action. Heavier smoking is more common among individuals treated with typical than atypical APDs, an observation that could lend support

to the anecdotal hypothesis that patients smoke to relieve extrapyramidal side effects of these drugs (Matthews et al. 2011; Tidey et al. 2005). Conversely, transition from a typical to an atypical APD, such as clozapine, has been found to reduce daily smoking, and clozapine treatment reduces NIC self-administration in rats, suggesting a potential effect on reinforcement (McEvoy et al. 1999; Abela et al. 2019). Atypical APDs have also demonstrated greater success in reducing both negative and positive symptoms than typical APDs, but have unique side effects that have also been associated with motivation to smoke (Matthews et al. 2011). Importantly, however, the high rate of smoking also observed in UHR individuals and first-degree nonpsychotic relatives, both of which experience select impairments observed in SCZ, suggests that APD side effects are likely not a primary driver of smoking (Ferchiou et al. 2012; Gupta and Mittal 2014). Nonetheless, medication status is an important variable to consider in studies of NIC use and effects in individuals with SCZ.

Because of the complexity of the association between symptom severity and smoking, experimental studies of the acute effects of NIC administration on measurable symptoms, such as cognitive impairments and sensorimotor gating, are valuable in studying the self-medication hypothesis. However, assessment of these effects is complicated by dependence and withdrawal in patients who smoke. Assessing the acute effects of NIC in NIC-naïve SCZ provides insight into improvements that could drive continuation of NIC use prior to the development of dependence. Multiple studies have suggested that acute NIC administration does enhance multiple areas of impaired cognitive performance in SCZ nonsmokers. For example, a single dose of transdermal NIC improved performance on a novelty detection task of episodic memory in nonsmoking patients (Jubelt et al. 2008). Another study of nonsmokers with SCZ and nonpsychiatric controls found that administration of transdermal NIC significantly improved attentional performance on

the Continuous Performance Test in both groups, and improved impulsive responding to a greater degree in SCZ subjects (R. S. Barr et al. 2008). However, an assessment of multiple indices of SCZ patients' cognitive function using the Repeatable Battery for Assessment of Neuropsychological Status (RBANS) before and after the use of NIC gum found improvement only in measures of attentional function in nonsmokers, with no benefit in smokers (Harris et al. 2004). This suggests that acute NIC administration may provide some initial cognitive benefits to SCZ patients, but these effects may diminish with repeated use as tolerance develops. Multiple studies have also found mixed effects of NIC on sensorimotor deficits in patients, including improvements in smooth pursuit eye movements but a lack of effect on auditory processing impairments in in both smoking and nonsmoking patients (Avila et al. 2003; Sherr et al. 2002; Inami et al. 2007; Inami and Kirino 2019).

Assessment of acute smoking status is imperative in interpreting measurements of cognitive and sensorimotor function before and after experimental NIC administration. Findings of baseline performance and NIC effects can vary greatly depending on whether smokers are assessed after normal smoking or during early withdrawal, which can begin within several hours of their last cigarette. This is key to understanding whether pro-cognitive effects of NIC observed in nonsmoking patients are present in smokers as well, or whether relative improvements in performance after smoking are a result of a "return to baseline" through relief from withdrawal-related impairments. Meta-analyses do provide some evidence that cognition-enhancing effects of acute NIC in control subjects may persist regardless of smoking status, but findings in patients are mixed (Heishman et al. 2010). When administered NIC through smoking or nasal spray prior to testing, SCZ smokers demonstrate increased levels of auditory PPI relative to both SCZ nonsmokers and briefly deprived smokers, indicating that the positive effects of acute NIC on PPI

can persist even after chronic smoking (Kumari et al. 2001; Woznica et al. 2009; Hong et al. 2008). However, similar studies suggest that improvement of PPI or MMN deficits by NIC administration in SCZ smokers may be limited due to a greater baseline impairment when measured after abstinence (George et al. 2006; Inami and Kirino 2019). Additionally, a finding of improved spatial working memory and attention in SCZ smokers after smoking may be more indicative of abstinence-induced exacerbation of these impairments than of the benefit of NIC administration (Sacco et al. 2005).

Though the effects of smoking on cognitive and sensorimotor gating deficits have been heavily studied as a means of examining the self-medication, studies directly assessing patients' motivations for smoking are a far less common, but useful, source of insight. These few reports suggest that addictive effects, anxiety, and reduction of negative symptoms are the most highly rated reasons for smoking among surveyed SCZ smokers (Gurpegui et al. 2007; Forchuk et al. 2002). Additionally, patients were more likely to cite stimulation and sensorimotor manipulation as motivators of smoking when compared to nonpsychiatric control smokers, though anxiolytic and addictive effects were more highly reported overall (A. M. Barr et al. 2008). These outcomes suggest potential, and possibly interacting, roles of both the reinforcement and self-medication hypotheses in driving high rates of smoking in SCZ.

1.4 STUDIES OF NICOTINE IN PRECLINICAL MODELS OF SCHIZOPHRENIA

Although SCZ is a disease distinct to humans, study of its pathophysiology in animal models is a valuable tool. SCZ is defined by its symptoms, as a clear understanding of underlying causes and pathology of the disease is lacking. Translational models of SCZ allow

for direct modeling of putative etiological factors, pathophysiological phenotypes observed in SCZ, and experimental study of behavioral outcomes. Thus, while there is no "ideal" animal model of SCZ that captures the full spectrum of related behavioral symptoms and known biological features, the goal of any model is to allow for the association of known neurobiological features of the disease with measurable behavioral parallels of symptoms in order to inform more effective treatment (Jones et al. 2011). Similarly, cigarette smoking is also unique to humans, but studies of NIC self-administration in animals have provided great insight into the reinforcing effects of NIC, as well as behavioral and biological factors that influence NIC use (O'Dell and Khroyan 2009). In both cases, animal models allow for a degree of control of factors such as age, sex, rearing environment, and drug exposure in experiments that is not feasible in clinical studies. Preclinical studies also circumvent challenging ethical limitations and expand the possibilities of experimental manipulation, as clinical studies ethically could not induce neurodevelopmental insult, perform invasive procedures to study SCZ neuropathology, or initiate regular NIC use in subjects. Animal models of psychiatric disease and drug abuse are therefore critical tools that further inform discoveries in clinical study.

A high priority in the preclinical study of NIC in SCZ is the use of an animal model that best captures known elements of disease pathophysiology as well as measurable and relevant behavioral and neurophysiological endpoints thought to be impacted by NIC intake. The majority of preclinical studies of NIC in SCZ models have focused on the "self-medication" model, exploring the effects of NIC and nicotinic drugs on SCZ-related behaviors and endophenotypes. For example, in adult offspring of dams treated with lipopolysaccaharide (LPS) during pregnancy, known as the maternal immune activation (MIA) model of SCZ, repeated administration of NIC

has been shown to reduce deficits in PPI and selective attention (Waterhouse et al. 2016). Subchronic treatment with ketamine induces severe disruption of prefrontal inhibitory activity and resulting cognitive impairments paralleling observations in SCZ, making ketamine treatment another popular rodent model of SCZ (Frohlich and Van Horn 2014). Treatment with NIC has been shown to normalize deficits in working memory (Rushforth et al. 2011) and multisensory integration (Jacklin et al. 2012) in ketamine-treated rats. However, preclinical studies exploring NIC reinforcement in animal models of SCZ are limited. A study of NIC self-administration the neonatal ventral hippocampal lesion (NVHL) model of SCZ suggested a modest increase in NIC intake among NVHL animals (Berg et al. 2014), though these results are complicated by the inclusion of daily administration of NIC or saline during the adolescent period. Additionally, when tested across a range of doses, MIA rats' responding for NIC did not differ from controls except during a brief phase of acquisition using a high response requirement. Total NIC intake and acquisition rates were also comparable between MIA and control animals (Waterhouse et al. 2018).

Our studies thus aimed to explore NIC reinforcement, as well as cognitive and neurophysiological effects of NIC, in a well-validated animal model of SCZ. The methylazoxymethanol acetate (MAM) rodent model is a neurodevelopmental model of SCZ that effectively captures numerous physiological perturbations observed in SCZ through administration of a mitotoxin (MAM) to pregnant dams on gestational day 17 (GD17) (Lodge 2013). Developmental disruption at this stage selectively impacts the developing paralimbic, temporal, and frontal cortices, producing a SCZ-relevant endophenotype in adult offspring. MAM animals display measurable positive symptom analogs, such as impaired PPI and hypersensitivity to psychostimulants such as amphetamine (Modinos et al. 2015), as well as cognitive impairments such as deficits in working memory, attentional processing, and behavioral flexibility (Lodge and

Grace 2009). Importantly, GD17 MAM also produces an endophenotype that elegantly recapitulates many neurophysiological disruptions observed in SCZ. Elevated striatal DA activity resulting from aberrant ventral hippocampal (vHipp) drive is a hallmark of the model and a useful mechanistic analog of psychosis (Modinos et al. 2015). Longitudinal PET imaging in patients correlates elevated striatal DA activity with progression of psychosis (Howes et al. 2011), and multiple lines of evidence implicate altered medial temporal lobe activity in driving striatal hyperactivity (Grace and Gomes 2019). MAM animals also demonstrate a reduction in cortical and hippocampal inhibitory interneuron function, and diminished hippocampal-prefrontal oscillatory activity reflecting generally disrupted E/I balance and connectivity (Lodge et al. 2009; Lodge and Grace 2007; Lodge and Grace 2009). Despite the high face and predictive validity of this model, the effects of NIC in the MAM model remain largely unexplored. One study of sensory gating deficits in MAM animals demonstrated limited positive effects of NIC administration on the rodent equivalent of human auditory P50 gating (Kohlhaas et al. 2015). Additionally, systemic or intra-vHipp administration of an α7 nAChR agonist has been shown to normalize elevated VTA DA population activity in MAM animals (Neves and Grace 2018). Our work thus aimed to more broadly assess the behavioral and neurophysiological effects of acute and chronic NIC in order to evaluate possible mechanisms behind heavy smoking in SCZ.

1.5 PURPOSE OF STUDIES

This dissertation summarizes a series of experiments that aimed to explore two potential hypotheses behind increased smoking in SCZ using the MAM rodent model. In **Chapter 2**, I explore the reinforcement-based hypothesis by comparing NIC self-administration between MAM

and control (CTL) animals across a number of doses, as well as dissociating the primary reinforcing and reinforcement-enhancing effects of NIC in this model. To our knowledge, this is the first study examining NIC self-administration in the MAM model. The experiments of **Chapters 3 & 4** focus on evaluating the self-medication hypothesis by assessing the effects of both acute and chronic NIC administration on several behavioral analogs of relevant SCZ symptoms and on activity of VTA DA and vHipp pyramidal neurons. In total, these experiments suggest that NIC is not more reinforcing in the MAM model of SCZ. However, they reveal that acute NIC does normalize both behavioral and neurophysiological dysfunction observed in the MAM model, lending potential support for smoking-induced mitigation of similar impairments in humans.

2.0 NICOTINE SELF-ADMINISTRATION IS NOT INCREASED IN THE MAM RODENT MODEL OF SCHIZOPHRENIA

2.1 INTRODUCTION

Despite success in reducing tobacco use in the general population over the last several decades, rates of smoking remain highly elevated among those with schizophrenia (SCZ). An estimated 60-80% of SCZ patients are smokers, as compared to 15-20% of the general U.S. population (de Leon and Diaz 2005; Dickerson et al. 2013). Smokers with SCZ smoke more heavily and more intensely, which contributes to negative health consequences in this population. Though this pattern of heavy smoking in smokers with SCZ is well-documented, no mechanistic explanation has yet been established. Two potential, but not mutually exclusive, hypotheses are that 1) nicotine (NIC), the primary reinforcing component of tobacco, mitigates some negative cognitive and/or sensory symptoms of SCZ, with smoking serving as a form of "self-medication", or 2) SCZ confers an increased magnitude of reinforcement derived from NIC.

Numerous clinical studies indicate that NIC administration to SCZ patients fails to impact positive psychotic symptoms but may be associated with improvements in cognitive deficits and negative symptom severity, contributing to the "self-medication" hypothesis (Smith et al. 2002; Beck et al. 2015). For example, one study found that both current and former smokers with SCZ showed higher performance in a neuropsychological assessment than SCZ "never-smokers", while control subjects did not vary by smoking history (Wing et al. 2011). However, evidence of improvement in negative symptoms and cognitive deficits, as well as alleviation of side effects from antipsychotic drugs, remains mixed. Conclusive study on the effects of NIC in SCZ patients

is complicated by several factors including differences in the withdrawal status and antipsychotic drug history of patients at the time of study. Many, but not all, studies require short-term or overnight abstinence prior to participation, which may contribute to variability in findings on the effect of NIC on cognitive function. For example, smoking after acute abstinence has been demonstrated to improve visuospatial working memory performance among SCZ patients (George et al. 2002; Sacco et al. 2005). However, other research suggests that the cognitive "costs" of NIC withdrawal and the relative benefits of NIC consumption are not specific to a psychiatric population. A study by AhnAllen and colleagues found that in both control and SCZ smokers, switching to very low nicotine content cigarettes reduced performance on measures of smokers' attention and visual processing, an effect mitigated by co-administration of NIC patch (AhnAllen et al. 2015). Other studies report no effects of early (1 day) or extended (3 week) NIC abstinence on attention or cognitive and behavioral tests in SCZ patients, suggesting NIC withdrawal may not have any effect on cognitive performance (Boggs et al. 2018; Hickling et al. 2018). Thus, while a "self-medication" hypothesis of smoking in SCZ is quite pervasive, experimental findings supporting this idea are limited.

Several lines of biological and behavioral evidence lend support to the hypothesis that NIC reinforcement is increased in SCZ individuals. The current dopamine hypothesis of schizophrenia posits that alterations in dopamine function lead to impairments in appropriately ascribing salience to relevant stimuli, which in turn contributes to dysfunction in motivated behavior and value learning (Howes and Kapur 2009; Juckel et al. 2006; Whitton et al. 2015). NIC-stimulated dopamine responses are thought to be increased in the mesolimbic dopamine system in SCZ, resulting in increased NIC reinforcement (Chambers et al. 2001; Dutra et al. 2012). Additionally, smokers with SCZ place higher incentive valuation on cigarettes than controls and are twice as

likely to select smoking over alternative reinforcers, such as money or candy, suggesting a particular sensitivity to smoking reinforcement(MacKillop and Tidey 2011; Spring et al. 2003). Limited impulse control may also drive persistent smoking among SCZ patients by undermining attempts to abstain from reinforced smoking behavior (O'Grada and Dinan 2007).

In addition to the broad range of clinical studies on smoking in SCZ subjects, preclinical work provides a valuable source of highly controlled research on the effects of NIC in SCZ and can address questions that cannot be approached in human subjects. Though evidence suggests some effect of acute NIC on cognitive performance in control animals, studies using animal models of SCZ have demonstrated limited support for increased cognitive or reinforcing effects of NIC in SCZ (Hernandez and Terry 2005; Rezvani and Levin 2001). For example, a study using the maternal immune activation (MIA) model of SCZ found that MIA animals showed modest improvements in a working memory task after extended NIC self-administration. However, MIA animals did not differ in self-administration from controls, except at a single dose with a high response requirement (Waterhouse et al. 2018). A study by Berg and colleagues using the neonatal ventral hippocampal lesion (NVHL) model of SCZ found that lesioned animals self-administer more NIC than controls only at a moderate dose of NIC (Berg et al. 2014). These limited positive results are complicated by the inclusion of daily s.c. injections of saline or NIC during the adolescent period. NVHL rats are particularly vulnerable to stress and stress associated with these injections might have differentially impacted NVHL and control rats (Tseng et al. 2008).

Thus, the data on whether NIC reinforcement is increased in animal models of SCZ are limited and unclear. The present studies examined NIC self-administration, the gold standard in studies of NIC reinforcement, in the model of SCZ produced by prenatal administration of methylazoxymethanol acetate (MAM), which is arguably the most comprehensive available rodent

model of SCZ (Ator and Griffiths 2003; Lodge and Grace 2009). The MAM model recapitulates many core features observed in SCZ and, compared to other rodent models of SCZ, consistently shows a high degree of both construct and predictive validity (Jones et al. 2011). Importantly, MAM treatment on gestational day 17 (GD17) has been consistently validated in numerous carefully controlled experiments both within and between labs (Modinos et al. 2015; Steeds et al. 2015; Grace and Gomes 2019). Adult MAM animals demonstrate physiological, pharmacological, behavioral, and anatomical perturbations consistent with observed SCZ phenotypes including amphetamine and phencyclidine hyper-responsivity, disrupted prepulse inhibition of startle and latent inhibition, thinning of cortical limbic structures, selective loss of parvalbumin-labeled GABAergic interneurons of the hippocampus, and hippocampal hyperactivity, as well as resulting increased dopamine population activity (Lodge and Grace 2009; Grace and Gomes 2019). Because of the critical role of dopamine neurophysiology in reward and reinforcement, as well as the high construct and predictive validity of this model, the MAM model is a strong yet previously unexplored paradigm to study questions of NIC reinforcement.

The following experiments thus sought to characterize NIC reinforcement in the MAM model of SCZ. Importantly, we aimed to study the primary reinforcing effects of NIC alone and in combination with its reinforcement-enhancing effects on other rewards. Although we hypothesized that MAM-treated rats would self-administer more NIC than control rats, the results show that in this model of SCZ there is no difference in NIC self-administration across a variety of doses and schedules of reinforcement, suggesting that SCZ pathophysiology modeled by MAM does not produce increased NIC reinforcement.

2.2 METHODS

Subjects. All experiments utilized Sprague-Dawley rats aged postnatal day (P)65-P75 at the start of experimental sessions. Rats were born in-house to timed pregnant dams (Envigo) injected intraperitoneally with saline (CTL; 1.0 mL/kg) or methylazoxymethanol acetate (MAM; 25.0 mg/kg, 1.0 mL/kg) on GD17 (Lodge 2013). After weaning on P22, litters were mixed and pups were separated by treatment group and sex. Initial studies were performed in female animals as males were used for separate sex-dependent experiments, but subsequent experiments used both males and females to examine potential sex differences (Experiment 3). Animals were housed in groups of 2-3 in tub cages with woodchip bedding in a ventilated rack until the time of surgery (P60-P70), after which they were housed individually. Each experiment utilized pups from at least 2 MAM-treated (MAM) and 2 saline-treated (CTL) litters and common features of the MAM phenotype (cortical thinning, reduced hippocampal volume) were confirmed in adult littermates through postmortem histological assessment. Additionally, some adult littermates were used in electrophysiological experiments and displayed characteristic increases in dopamine neuron population activity (Grace and Gomes 2019). Animals had *ad libitum* access to water in the home cage throughout the duration of the experiment and began food restriction at least 48 hours prior to the first experimental session unless otherwise noted. Rats were allotted 20 grams (males) or 15 grams (females) of chow (LabDiet Rodent 5001; LabDiet, St. Louis, MO) daily to maintain body weight at approximately 80% of free-feeding weight. Facilities were maintained on a reversed 12-hour light-dark cycle (lights off 0700), and all experimental procedures were carried out during the dark phase. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Apparatus. All experimental sessions were conducted in 38 operant chambers (30.5 cm × 24.1 cm × 21.0 cm; ENV-008CT; Med-Associates, Fairfax, VT) enclosed in sound-attenuating cubicles with a ventilation fan. Operant chambers were outfitted with two nosepoke holes (2.5 cm in diameter), spaced 14 cm apart. White cue lights (3.5 cm in diameter) were located 6.25 cm above the top of each nosepoke portal. A houselight located 1 cm below the ceiling of the chamber in the center of the wall containing the nosepoke portals was covered with a red filter sleeve except in experiments using a reinforcing visual stimulus (VS; see Experiment 3). Intravenous (i.v.) infusions were delivered via an infusion pump through tubing connected to each animal's catheter. Tubing was protected by a flexible metal spring casing connected to a swivel system that allowed for largely unrestricted movement.

Nicotine. Nicotine hydrogen tartrate salt was dissolved in 0.9% sterile saline and passed through a 0.22 μM filter to ensure sterility. Nicotine solutions were made every 2-4 weeks and stored at 4° C until experimental use. Syringes for self-administration sessions were freshly made each day. Doses are expressed as free base.

Surgery and maintenance of catheter patency. Surgery was performed between P60-70. Rats were anesthetized using isoflurane (2%-3% in 100% O2) and implanted with indwelling catheters into the right jugular vein (Donny et al. 1999). Ports were capped with plastic tubing and flushed daily with 0.1 mL heparinized (30 U/mL) saline containing streptokinase (9.333 U/mL) and gentamicin (66.67 mg/mL) for 5-6 days of recovery before self-administration. During the experimental period, catheters were flushed with 0.1 mL heparinized saline before and 0.1 mL heparinized saline with gentamicin (66.67 mg/mL) after each self-administration session. Catheter patency was confirmed via rapid loss of muscle tone after infusion of methohexital (5 mg/kg) following the final self-administration session at each dose and the final

self-administration session of each experiment. Rats that failed a patency test (1 – 4 rats per experiment) were excluded from data analysis.

Self-administration sessions. Self-administration sessions took place for 1 hr daily within the first several hours of the dark cycle (except where otherwise noted). One nosepoke portal was assigned as active and resulted in the delivery of an i.v. infusion in approximately 1 second at a volume of 0.1 mL/kg after meeting the fixed-ratio (FR) response requirement, followed by a 60 s timeout period during which responses were recorded but not reinforced. As noted in each experiment, infusions were paired with either a neutral cue (10 s illumination of the cue light above the active nosepoke) or a reinforcing VS (1 s illumination of cue light, followed by 1 min extinction of house light and cue light). Prior studies have consistently demonstrated that rats will respond for the VS when presented alone, and this responding is enhanced by nicotine (Donny et al. 2003). Conversely, rats do not respond to the 10 s illumination of the cue light alone, demonstrating that it is not inherently reinforcing (Rupprecht et al. 2015). It can, however, become a conditioned reinforcer when associated consistently with an unconditioned reinforcer, including long-term pairing with high doses of nicotine (Palmatier et al. 2008). Infusion delivery was adjusted daily for body weight. Responses at the second, inactive nosepoke portal were recorded but had no consequence. Left and right nosepoke portals were randomly assigned as active and inactive. In applicable experiments, criteria for acquisition of NIC self-administration on an FR2 schedule were an average of 5 or more earned infusions over a 3-day period and twice as many active responses as inactive per session during that period.

*Statistical analyses***.** Average responding over the final three days of each dose or response requirement was analyzed for each experiment; values are reported as mean ± SEM. ANOVA tests with repeated measures (Dose or Day, where appropriate) were followed by post-

hoc tests (α =0.05; reported p values are adjusted for multiple comparisons using Bonferroni correction) comparing by Group (MAM and CTL). Data analyses were conducted in IBM SPSS Statistics and GraphPad Prism.

2.3 EXPERIMENTS

Experiment 1: Primary reinforcing effects of NIC in MAM and CTL animals. In order to assess primary NIC reinforcement in MAM (n = 22; 4 litters) and CTL (n = 17; 4 litters) animals, female rats were allowed to respond for infusions of NIC paired with a neutral cue (10-s illumination of cue light above active nosepoke). Rats were allowed to respond for 60 µg/kg/infusion on a FR2 schedule for 10 days. The schedule was then changed to FR5, and nicotine dose was reduced every 5 days (60, 15, 7.5, 3.75 µg/kg/infusion).

Experiment 2: Responding for NIC in extended-access sessions. One-hour self-administration sessions may fail to capture differences in behavior that may emerge over an extended period of access to NIC, as well as potential changes in self-administration over the light-dark cycle as a result of MAM administration. To examine differences in NIC intake within extended access sessions and patterns of NIC-taking across the light-dark cycle, female MAM (n = 8; 2 litters) and CTL (n = 6; 2 litters) animals were allowed to self-administer NIC (30 µg/kg/infusion) in 23-hr self-administration sessions (11 hr lights-off, 12-hr lights on) through nosepoke responding on a FR2 schedule. Animals were not food restricted and had ad libitum access to water and to chow pellets on a FR1 schedule via responding at a single lever on the wall opposite the nosepokes. Animals were returned to tub cages for one hour during the dark phase each day (1000-1100 h) for cleaning of operant chambers.

Experiment 3: Dual-reinforcing effects of NIC in MAM and CTL animals. NIC acts as a primary reinforcer on its own and potently enhances the reinforcing value of other rewards (Donny et al. 2003). Indeed, non-pharmacological stimuli accompanying NIC play a critical role in driving NIC self-administration and little is known about the nicotine enhancement of non-drug reinforcers in the SCZ population. In order to examine both primary reinforcing and reinforcement-enhancing effects of NIC in MAM and CTL animals, MAM (n = 9 males, 13 females, 7 litters) and CTL (n = 10 males, 11 females, 7 litters) rats were allowed to respond as described above for infusions paired with a VS. All sessions began with the white houselight illuminated. Completion of the response requirement produced a 1-s illumination of the cue light above the active nosepoke portal followed by a complete lights-off lasting 60 s. During this time, responses at the active nosepoke were recorded but produced no consequence. Animals were allowed to acquire responding for VS paired with infusions of saline on an FR2 schedule. Dose of NIC was increased every 7 days from 0, 3, 10, 30, 100, and 150 µg/kg/infusion.

Experiment 4: Sucrose reinforcement in MAM and CTL animals. In order to compare responding for a natural reinforcer in MAM and CTL animals, female MAM (n = 12, 3 litters) and CTL rats (n= 14, 3 litters) were allowed to respond for 45 mg sucrose pellets (Bio-Serv, Flemington, NJ) in daily 1-hr sessions on FR1 (2 days), FR5 (2 days), and then FR10 (3 days) schedules, before completing six days on a progressive-ratio (PR) schedule of responding in which the response requirement increased with each successive reinforcer delivered.

2.4 RESULTS

Experiment 1: MAM and CTL animals do not differ in NIC primary reinforcement across doses. MAM and CTL rats reliably self-administered NIC, responding more at the active portal than the inactive (Figure 1A). There was a significant effect of Dose ($F_{3,35} = 8.47$, $p < 0.001$), but no effect of Group ($p = 0.886$) and no interaction of Dose x Group ($p = 0.913$) in NIC self-administration. Both MAM and CTL animals demonstrated a characteristic inverted-U dose-response relationship but did not differ significantly in infusions earned at any dose (Figure 1B). MAM animals required significantly longer to meet acquisition criteria for NIC self-administration (8.7 ± 1.5 days versus 4.2 ± 0.6 among CTL animals, $p < 0.01$).

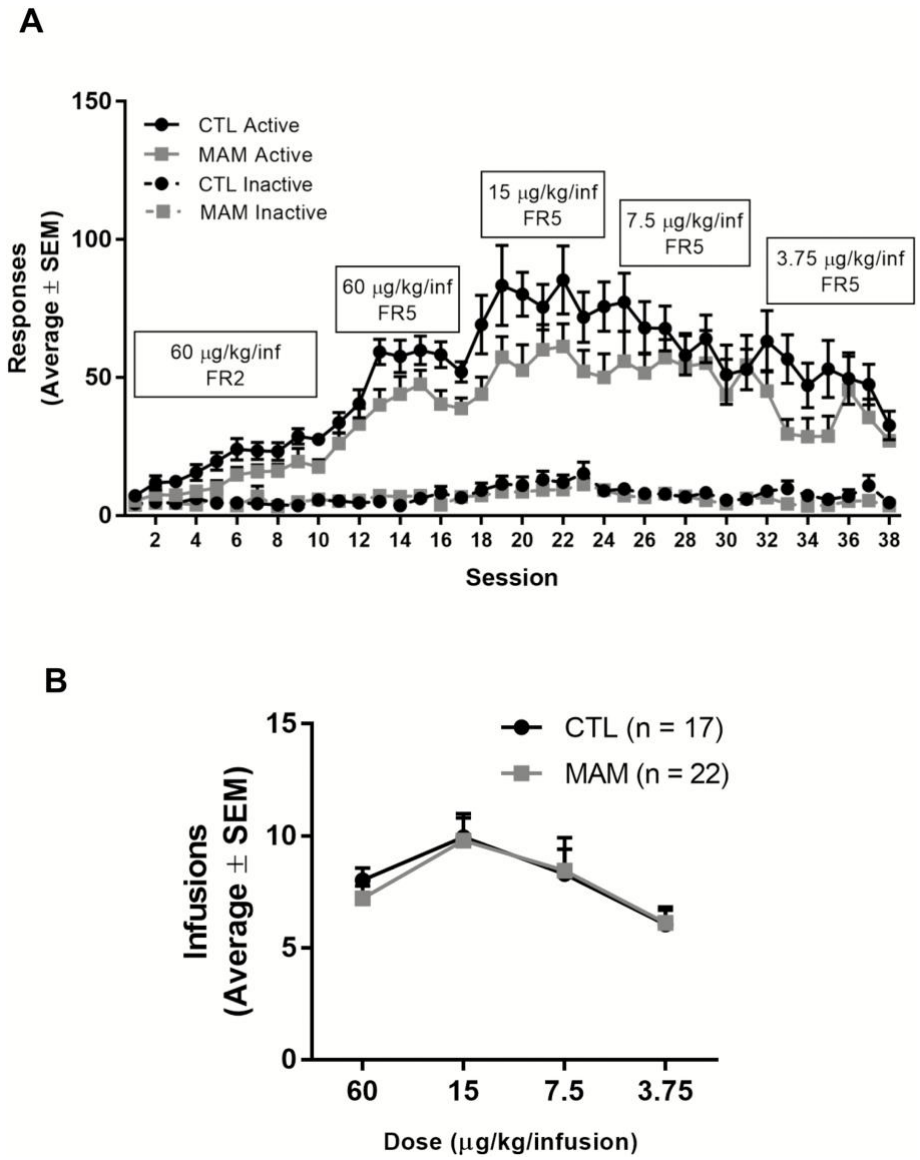

Figure 1: Nicotine primary reinforcement is comparable in MAM and CTL animals

(A) Average responding for NIC at the active and inactive nose-poke portals. MAM and CTL animals reliably acquired NIC self-administration, responding more at the active than inactive nose-poke. (B) Average infusions earned by MAM and CTL rats at each NIC dose. Data points express the mean number of infusions earned over the last three sessions at each dose. MAM and CTL animals did not differ significantly at any dose tested. CTL, control; MAM, methylazoxymethanol acetate; NIC, nicotine.

Experiment 2: MAM and CTL animals do not differ in NIC self-administration in an extended-access paradigm. Compared to CTL rats, there was no impact of MAM on nicotine self-administration in 23-h extended access sessions (Figure 2A-B). There was no significant effect of Day ($F_{20,251} = 1.085$, $p = 0.37$) or of Group ($F_{1,251} = 0.91$, $p = 0.34$) on mean infusions earned. Additionally, MAM and CTL rats did not differ in patterns of responding within each session (Figure 2C). Within the light-dark cycle of a single session, there was a significant effect of Light on responding ($F_{1, 24} = 31.26$, $p < 0.001$), as both MAM and CTL animals responded markedly less during the light phase. MAM and CTL animals did not differ in mean food pellets earned per session over the final three experimental sessions (MAM = 535.0 ± 15.0, CTL = 548.6 ± 7.6 pellets; $p = 0.18$).

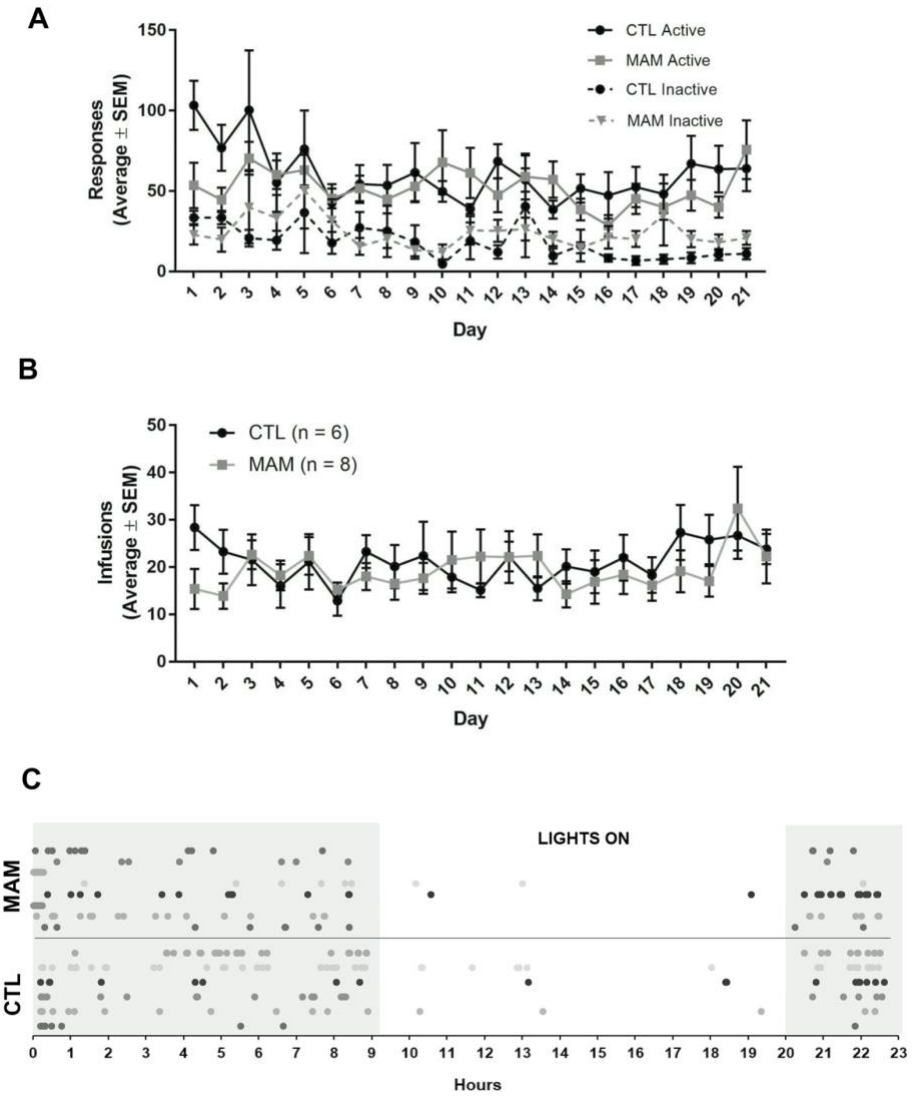

Figure 2: MAM and CTL animals do not differ in self-administration over an extended access period

(A) Average responding for NIC at the active and inactive nose-poke portals over each 23-hour session. Both MAM and CTL animals reliably responded more at the active than inactive portal. (B) Average infusions earned across all 23-hour access sessions. MAM and CTL animals did not differ significantly in the number of infusions earned in each session across the 21 days of self-administration. (C) Representative infusion data for a single 23-hour access session (day 17). Each row of data points represents the infusions earned by one rat across the 23 hours of self-administration, wherein each point is a single infusion. Shaded boxes represent the dark phase of the session. MAM and CTL animals did not demonstrate any differences in patterns of responding across the 23-hour access period.

Experiment 3: MAM animals of both sexes respond less for NIC paired with a reinforcing VS. Both male and female MAM rats responded less than controls for NIC paired with a mildly reinforcing light stimulus (Figure 3). There was a significant effect of Group ($F_{1, 242} = 40.74$, $p < 0.001$) and of Dose ($F_{4, 242} = 47.11$, $p < 0.001$), as well as a Group x Dose interaction ($F_{4,242} = 4.88$, $p < 0.005$). There was no effect of Sex ($p = 0.155$) or interaction of Sex x Group ($p = 0.88$), Sex x Dose ($p = 0.66$), or Sex x Group x Dose ($p = 0.91$). MAM animals earned significantly fewer VS presentations than CTL animals when paired with saline or with 3, 10, 30, or 150 μg/kg nicotine infusions ($ps < 0.05$). There was no effect of Sex ($p = 0.25$), Group ($p = 0.71$), or Sex x Group ($p = 0.40$) on days to meet acquisition criteria. Across doses, MAM and CTL animals made an average of 5 or fewer inactive nosepoke responses per session and at least twice as many active as inactive responses.

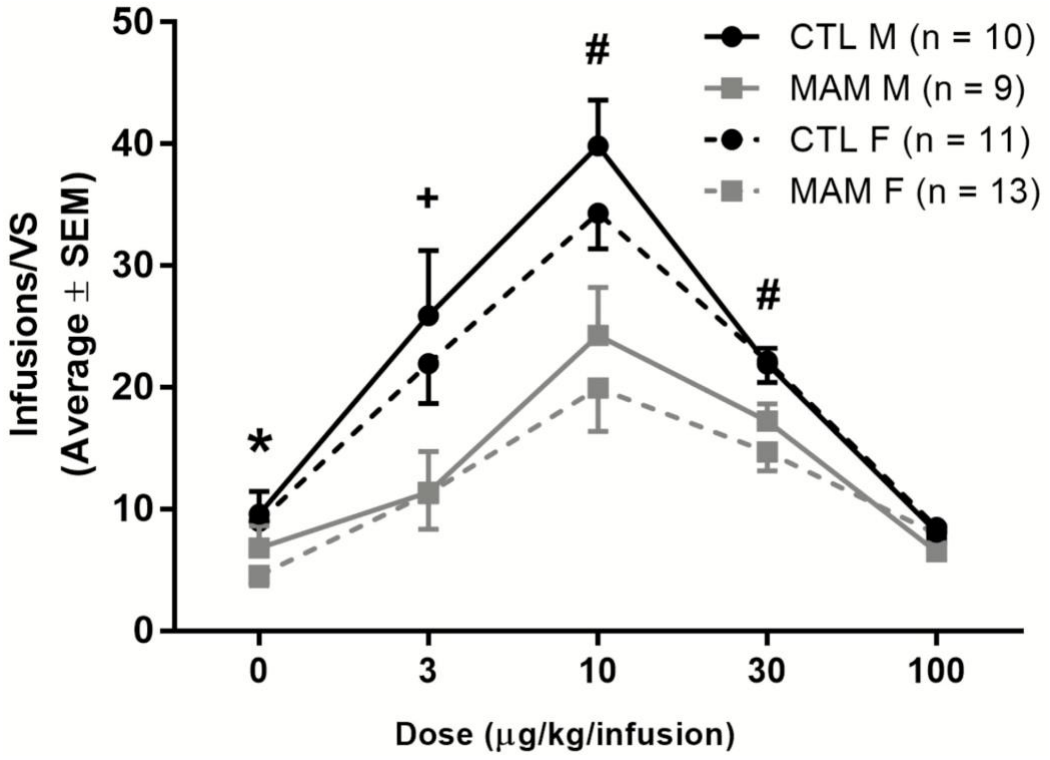

Figure 3: CTL animals self-administer more NIC infusions + VS than MAM animals

Average infusions + VS earned by MAM and CTL rats of each sex at each NIC dose. Data points express the mean number of reinforcers earned over the last three sessions at each dose. CTL animals earned significantly more reinforcers than MAM animals at 0 µg/kg/inf (*$p < .05$), 3 µg/kg/inf (+$p < .005$), 10 µg/kg/inf (#$p < .001$), and 30 µg/kg/inf (#$p < .001$), but did not differ significantly at 100 µg/kg/inf ($p = .164$). There was no effect of sex at any dose ($p = .611$).

Experiment 4: Sucrose reinforcement is reduced in MAM animals. Average responses for sucrose pellets on a PR schedule of reinforcement over the final three days of the experiment were compared between MAM and CTL animals. MAM and CTL animals made comparably low (<15) inactive responses per session, but MAM animals responded significantly less than CTL animals at the active nosepoke ($t_{24} = 2.25$; $p < 0.05$, unpaired t-test; Figure 4) and reached lower breakpoints, suggesting that sucrose rewards are less reinforcing to MAM rats than CTL.

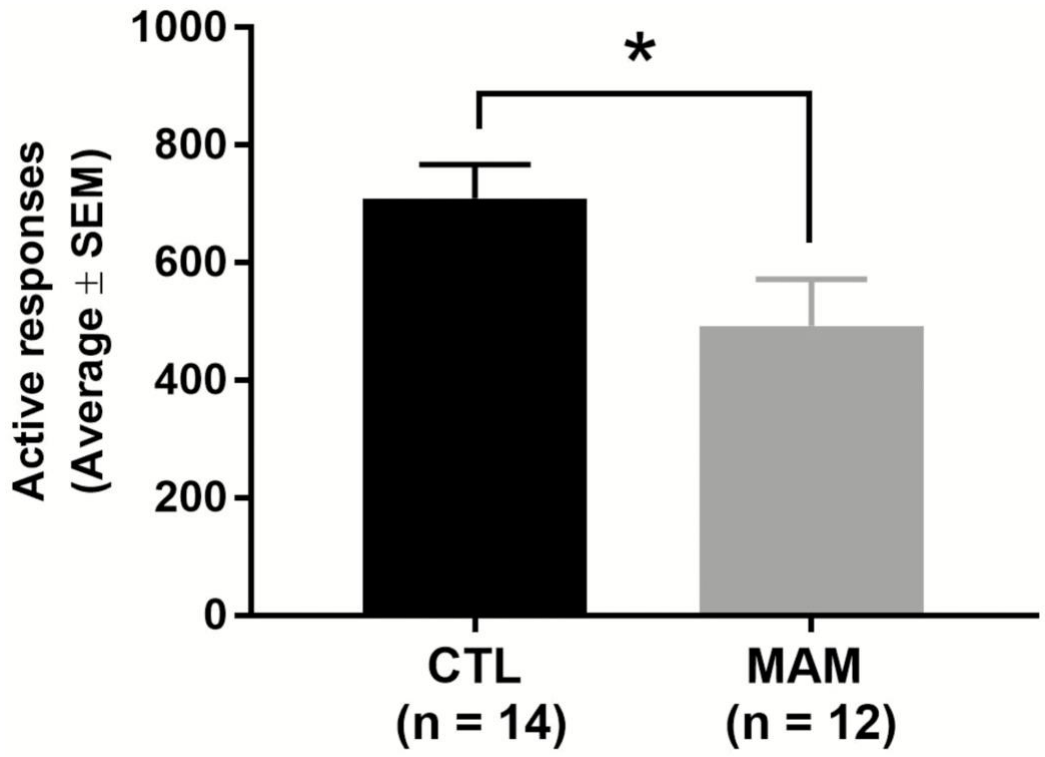

Figure 4: MAM animals respond significantly less for sucrose rewards than CTL animals

Average active and inactive responding for sucrose pellets on a progressive ratio schedule of reinforcement. Bars represent average responses made over the final 3 days of testing. CTL animals responded significantly more than MAM animals at the active nose-poke ($t_{30} = 2.19$; $p < .05$; significant difference indicated by *).

2.5 DISCUSSION

The primary reinforcing action of NIC is not increased in the MAM model of SCZ. The present study utilized the MAM model of SCZ, which is arguably the most comprehensive rodent model of SCZ currently available[35,36], to study nicotine reinforcement. The results of our experiments demonstrate that MAM-treated rats do not differ from controls in the primary reinforcing effects of NIC. Both groups responded comparably for NIC across a range of doses in 1-hr sessions. Moreover, when rats were allowed to self-administer NIC in 23-hr access sessions, MAM-treated and CTL animals did not differ in infusions earned across sessions or in their patterns of responding across the light-dark cycle within each session. These findings suggest that E17 MAM administration does not promote increased NIC intake in both short and long access periods.

Combined primary reinforcing and reinforcement enhancing effects of NIC are reduced in MAM-treated animals. In the presence of non-NIC reinforcers, NIC self-administration is driven by the combined actions of NIC as a primary reinforcer, as well as its enhancing effects on reinforcement from other rewards. In our experiments, MAM rats responded significantly less for NIC paired with a reinforcing VS relative to controls across several NIC doses, a measure that reflects a reduction in the combined primary reinforcing and reinforcement enhancing effects of NIC. However, comparing the reinforcement enhancing action of NIC between MAM-treated and CTL rats is complicated by the observation that MAM-treated animals responded less than controls for VS reinforcers even in the absence of nicotine. The reinforcement enhancing effect of NIC is correlated with the reward strength of the reinforcer that is enhanced. Thus, one potential interpretation of these results is that NIC reinforcement enhancement, like primary reinforcement, is similar between MAM-treated and CTL animals, as MAM-treated animals responded less for

VS alone but showed a proportionally similar magnitude of enhanced responding when reinforcers were delivered with NIC. Additionally, while previous studies have reported small sex differences in self-administration at certain NIC doses, key differences in design between those and the present study may account for the lack of sex differences observed here (Rupprecht et al. 2015).

Reinforcing effects of NIC relative to other rewards in MAM-treated animals. The reduced level of responding for VS or sucrose among MAM animals suggests features of anhedonia consistent with those observed in SCZ (Whitton et al. 2019). In contrast, MAM and CTL animals responded comparably for NIC alone. This might suggest that NIC is more reinforcing than other rewards to MAM-treated animals, but is not more reinforcing in MAM-treated rats compared to CTL animals. Indeed, there is an observed relationship between severity of anhedonia and smoking status in SCZ, and many SCZ smokers cite positive affective effects of NIC particularly when they are feeling "low" (Khantzian 2016; AhnAllen et al. 2012). Interestingly, other studies employing the MAM model have found inconsistent results in reward-related behavior. One study found that MAM-treated rats consistently pursued the highest reinforced option in a behavioral flexibility task, and made fewer perseverative choices compared to CTL animals (Kaneko et al. 2017). Though this reported reduction in perseverative behavior is counter to previous observations in MAM-treated animals, the tendency to choose the more highly reinforced option may suggest an increased sensitivity to natural rewards (Gastambide et al. 2012). While there are no previous reports of NIC self-administration in MAM-treated animals, a study by Ruda-Kucerova and colleagues demonstrated that MAM-treated animals did not differ from controls in methamphetamine self-administration, despite a link between SCZ and methamphetamine use in clinical populations (Ruda-Kucerova et al. 2017). It is important to note, however, that complex variables beyond reward, such as stress and environmental factors, likely interact with

neurobiological factors to influence smoking in SCZ patients, and should be considered when studying the issue as a whole (Prochaska et al. 2017)

Studies of NIC in other animal models of SCZ. Although this is the first study of NIC self-administration in the MAM rodent model of SCZ, published studies using other rodent models of SCZ report mixed results. The NVHL model, for example, was found to produce a modest increase in responding for a moderate dose of NIC + VS, but not higher or lower doses, and an increase in responding during extinction and reinstatement of NIC + VS self-administration (Berg et al. 2014; Rao et al. 2016). NIC self-administration did not improve performance in a spatial learning and working memory task in NVHL or sham animals, suggesting a lack of cognitive effect (Berg et al. 2014). However, these studies are potentially confounded by the stress of repeated injections during adolescence prior to NIC self-administration. Conversely, pharmacological models of SCZ such as phencyclidine (PCP) administration and amphetamine sensitization, which aim to capture elements of hyperglutamatergic and hyperdopaminergic states in SCZ respectively, consistently failed to increase NIC self-administration in rats, and have even demonstrated a reduction in NIC + VS self-administration relative to controls (Fletcher et al. 2018; Swalve et al. 2015). Moreover, while the MIA model also did not alter NIC self-administration, NIC self-administration did mitigate cognitive deficits observed in MIA animals, lending further support for a self-medication hypothesis (Waterhouse et al. 2018). Additionally, mice with a mutation commonly observed in human SCZ patients in CHRNA5, which codes for the α5 subunit of the nicotinic receptor, demonstrated cortical hypofunction that could be normalized with chronic NIC delivery (Koukouli et al. 2017). This diversity of findings may reflect differences in the elements of SCZ pathophysiology captured by each particular model and their impact on the effects of NIC, as well as limitations in certain experimental designs. The MAM model is distinct from other animal

models of SCZ in its encapsulation of behavioral, anatomical, pharmacological, and physiological perturbations observed in the disease, namely those of the midbrain dopamine system, achieved through neurodevelopmental disruption. The overall impression conferred by our findings in the MAM model as well as those in observed in several different models of SCZ is that NIC self-administration is not consistently or robustly exaggerated, in contrast to the increased smoking seen among SCZ patients. These findings are consistent with the hypothesis that the reinforcing effects of NIC are not altered in SCZ. Thus, this suggests that increased smoking is not driven by increased NIC reinforcement but rather by another motivating factor, such as improved cognitive or social functioning, that is not captured in animal self-administration studies.

Differences in valuation of cigarettes vs. non-NIC rewards observed in SCZ smokers. The findings of our experiments align with some aspects of NIC use and smoking in human SCZ patients. While we did not observe increased NIC intake among MAM-treated animals, which would parallel heavier smoking in SCZ patients, our results reflect a difference in the relative valuation of NIC vs. non-NIC rewards that may also be observed clinically. Studies of human patients completing tasks to earn chocolate rewards have also suggested that observed deficits in goal-directed learning are not due to insensitivity to reward value or reward prediction error (Morris et al. 2018). However, studies of SCZ smokers have suggested a specific overvaluation of cigarette reinforcement. SCZ smokers report greater hedonic response to NIC than control smokers, and SCZ smokers select cigarettes over alternative reinforcers twice as often as controls in a hypothetical choice task (Spring et al. 2003; Tidey 2016). Such findings provide an alternative hypothesis to the notion that smoking serves as a form of "self-medication" of aberrant sensory and cognitive symptoms and has more robust pro-cognitive effects in SCZ than in controls, which has also been challenged by direct studies (Hahn et al. 2013). Thus, our results in the MAM model

may parallel differences in the valuation of cigarettes relative to other reinforcers observed in human subjects to some degree, though direct evaluation of reinforcing efficacy could be better studied through experiments assessing elasticity of demand or choice procedures between NIC and other reinforcers. Regardless of experimental approach, however, concomitant use of antipsychotic drugs in patients within human studies is an additional difference between clinical and preclinical results, as varying side effects of these drugs could influence NIC effects, including reinforcement (Wijesundera et al. 2014).

Final conclusions. Overall, these findings suggest that SCZ pathophysiology, as modeled by E17 MAM administration, does not produce an increase in NIC reinforcement. To the extent that MAM-treated rats are a valid model of SCZ, these results suggest that increased NIC reinforcement does not account for increased smoking in SCZ patients. Despite modeling many neurobiological aspects of SCZ, the MAM model fails to recapitulate SCZ entirely, and thus, alternatively, could fail to capture a specific biological or symptomatic feature that would increase NIC reinforcement. While our results do not directly follow the increased smoking observed in clinical SCZ populations, the differences observed in MAM-treated animals' responding for NIC relative to other rewards may have relevant implications. Smokers with SCZ are a vulnerable sub-population that may be differentially affected by tobacco control policies, such as mandated reduction of NIC content in tobacco products, and a more thorough understanding of NIC behavioral pharmacology in this population would be helpful in informing proposed policies. Further clinical study is necessary to better understand motivators and outcomes of smoking in SCZ, which can be supplemented by preclinical exploration of mechanistic solutions.

3.0 NICOTINE MITIGATES SOME COGNITIVE IMPAIRMENTS IN THE MAM MODEL OF SCZ

3.1 INTRODUCTION

An elevated rate of smoking among those with schizophrenia (SCZ) remains a persistent issue contributing to discrepancies in health and quality of life. Despite long-established knowledge of this high incidence, the mechanism driving this particularly heavy smoking remains unknown. The use of nicotine (NIC), the primary reinforcing component of tobacco, as a form of self-medication among SCZ smokers is one prevailing hypothesis. Anecdotally, this self-medication hypothesis was long associated with relief of antipsychotic drug side effects (Goff et al. 1992). However, elevated rates of tobacco use are observed before psychosis onset and when medication status is controlled for, challenging this explanation as a primary motivator of heavy smoking (Ward et al. 2019; de Leon and Diaz 2005). A great deal of research has also focused on the direct effects of NIC on sensory gating and cognitive impairments in SCZ, with the hypothesis that smoking provides some relief of SCZ-related dysfunction, which drives continued tobacco use (Kumari and Postma 2005).

In order to explore the validity of the self-medication hypothesis, many studies have attempted to assess the effects of NIC on measures of cognitive dysfunction in SCZ patients. Comparison of cognitive symptom severity in SCZ smokers versus nonsmokers may not be an ideal metric to inform the self-medication hypothesis, as one cannot determine whether being a smoker impacts cognitive status, or if those with more severe symptoms are more likely to smoke (Wing et al. 2011; Taiminen et al. 1998). Because of this, experimental assessments of NIC effects

on cognition in both smokers and nonsmokers are valuable. Acute transdermal NIC administration can improve attentional performance, response inhibition, and episodic memory in SCZ nonsmokers (R. S. Barr et al. 2008; Jubelt et al. 2008). Cigarette smoking has also been demonstrated to improve visuospatial working memory (VSWM) performance in SCZ smokers, while acute smoking abstinence selectively exacerbates impairments in this group (Sacco et al. 2005; George et al. 2002). Differences in the effects of NIC on attention in nonsmoking versus smoking subjects may suggest a development of tolerance or desensitization to its cognition-enhancing effects. For example, NIC gum selectively improved attention in nonsmoking, but not smoking, SCZ patients, with no effects on any other cognitive domains assessed using the testing battery (Harris et al. 2004). Conversely, however, assessments of attentional performance in studies comparing controls and SCZ patients suggest that NIC improves attention in both SCZ smokers and nonsmokers, while only nonsmoking controls demonstrate NIC-induced improvements (R. S. Barr et al. 2008; Sacco et al. 2005). These findings illustrate the importance of considering effects of acute NIC administration in both nonsmokers and smokers, as well as the effects of acute smoking abstinence, in clinical studies of cognition.

Experimental NIC administration also consistently improves sensory gating deficits in SCZ subjects. Sensory gating is a key neurological process that functions to filter redundant or unnecessary stimuli from higher cortical processing, effectively preventing an excess of irrelevant environmental stimuli. Deficits in sensory gating measured by assessments such as tactile or auditory prepulse inhibition of startle (PPI) or specific auditory evoked potentials (AEPs) in response to successive stimuli are a reliable clinical marker of SCZ. This complex mechanism arises from a precise balance of excitatory and inhibitory signaling throughout multiple brain regions, including hippocampus, thalamus, and prefrontal cortex (Swerdlow et al. 2016). The

severity of these deficits may therefore be associated with other cognitive symptoms of the disease, as they are indicative of a wider systemic impairment of inhibition and filtering of unnecessary stimuli (Xia et al. 2020). NIC administration consistently enhances auditory and tactile PPI in both control and SCZ smokers and nonsmokers, suggesting a direct pharmacological effect of NIC rather than a reversal of withdrawal-induced deficits (Postma et al. 2006; Kumari et al. 1997; Hong et al. 2008; Kumari et al. 1996). When measured under conditions of smoking satiation, SCZ smokers were comparable to control smokers in levels of PPI, suggesting that normalization of PPI in SCZ is maintained by smoking (Woznica et al. 2009). Additionally, sensory gating impairments measured by P50 auditory evoked potentials are less pronounced in non-deprived SCZ smokers than in SCZ nonsmokers, suggesting that NIC may have a persistent effect on sensory gating in SCZ smokers (Chen et al. 2011). In fMRI studies, NIC also improved auditory selective attention and decreased task-associated neuronal hyperactivity as measured by BOLD response in a group of SCZ mixed smokers and nonsmokers (Smucny et al. 2016).

Importantly, a great deal of evidence implicates α7 nAChRs in regulating processes underlying both sensory gating and cognitive performance, an association that likely provides insight into the procognitive effects of NIC (AhnAllen 2012). Alterations in CHRNA7, the gene coding for the α7 subunit, are consistently linked with both smoking and cognitive and sensory gating deficits in SCZ, and α7 nAChRs are significantly reduced in several brain regions in SCZ (Leonard et al. 2002; De Luca et al. 2004). This receptor has been of great interest in development of potential therapies for these impairments in SCZ, though no compounds have proceeded successfully through clinical trials (Tregellas and Wylie 2019). Although α7 receptors have a relatively low affinity for NIC, the high blood NIC levels attained by intense patterns of smoking in SCZ may be particularly effective in activating these receptors.

Although SCZ is a distinctly human illness characterized by constructs that are not possible to measure in non-human subjects, animal models capturing aspects of the disease are useful in developing a mechanistic understanding of symptoms and possible treatments. Studies in animal models allow for a degree of control over factors such as drug history, smoking status, and lifestyle that is difficult or impossible to achieve with human subjects. Additionally, studies in animal subjects allow researchers to pursue invasive or potentially risky approaches that would be ethically challenging in clinical studies. While animal models can vary widely in induction method and resulting phenotypes, preclinical studies of the effects of NIC and nicotinic agonist drugs on cognitive performance have thus far yielded results largely parallel to those in clinical studies. Both NIC and α7 agonists have been shown to enhance performance on cognitive flexibility and reversal learning phases of the attentional set-shifting task (ASST) in control rats, though studies of drug effects on this task in model animals are limited (Allison and Shoaib 2013; Wood et al. 2016). Chronic NIC, both self-administered and experimenter-administered, normalized PPI and improved latent inhibition, a measure of selective attention, in the maternal immune activation (MIA) rodent model of SCZ (Waterhouse et al. 2016; Waterhouse et al. 2018). Additionally, acute administration of NIC normalized deficits in a novel multisensory integration task in rats treated with ketamine, a common pharmacological model of SCZ (Cloke et al. 2016). However, acute NIC produced only modest improvements in performance of the radial-arm maze task in the neonatal ventral hippocampal lesion (NVHL) model (Berg et al. 2014). Variation in findings across different assessments, NIC administration schedules, and animal models illustrate the need for the study of both acute and chronic effects of NIC on a range of tasks in a well-validated model of SCZ.

The methylazoxymethanol acetate (MAM) rodent model of SCZ is a neurodevelopmental disruption model with high predictive and face validity (Lodge and Grace 2009). Administration of MAM on gestational day (GD) 17 to pregnant rats produces reliable, measurable behavioral deficits in adult offspring that parallel human cognitive dysfunction seen in the disease. For example, offspring of MAM-treated dams, referred to herein as MAM animals, consistently demonstrate impairments in short-term memory as measured by the novel object recognition (NOR) task, as well as deficits in working memory and reversal learning in assessments such as the Y-maze and ASST (Featherstone et al. 2007; Lodge and Grace 2009). Disruption of several measures of sensory gating have also been documented in this model, including reductions in auditory PPI, N40 auditory evoked potential (a rodent equivalent to the human P50 sensory gating response), and mismatch negativity (MMN) responses (Moore et al. 2006; Kohlhaas et al. 2015). To date, studies of the effects of NIC and nicotinic drugs on dysfunction in the MAM model have been limited. Kohlhaas et al. (2015) reported modest improvements in the N40 potential and MMN response in MAM animals after acute treatment with NIC or the α7 nAChR agonist drug ABT-107 (Kohlhaas et al. 2015). Additionally, administration of both full and partial α7 agonists has been shown to normalize increased VTA DA population activity in MAM animals via action in ventral hippocampus (vHipp) (Neves and Grace 2018). This finding could suggest a potential mechanism through which NIC may affect behavioral impairments observed in the MAM model.

The present studies aimed to evaluate the effects of both acute and chronic NIC treatment on assessments of cognitive function and sensory gating in the MAM rodent model of SCZ. These experiments allow the important distinction of determining how an acute dose of NIC may differently affect behavior in NIC-naïve subjects versus those chronically exposed to and briefly abstinent from NIC. This is intended to more closely model experiments of NIC effects on human

smokers, who may be tested after overnight abstinence as a baseline and then again after NIC administration. In our study, assessments were performed at both a Pre-NIC Baseline and a Post-NIC timepoint within each group of subjects when possible. We measured auditory PPI in MAM and CTL rats before and after NIC administration to determine how the drug may affect sensory gating. In addition, the effects of NIC on episodic and working memory and cognitive flexibility were assessed using a NOR task and an attentional set-shifting task (ASST), which are relevant analogs of human behavioral tests and are well-established in the MAM model of SCZ. We hypothesized that acute administration of NIC, both in NIC-naïve and chronically-treated animals, would improve deficiencies in these measures in MAM animals relative to a Pre-NIC Baseline.

3.2 MATERIALS AND METHODS

Animals. All experiments utilized Sprague-Dawley rats aged 2 – 6 months at the start of experimental sessions. Rats were born in-house to timed pregnant dams (Envigo) injected intraperitoneally with saline (CTL; 1.0 mL/kg) or methylazoxymethanol acetate (MAM; 25.0 mg/kg, 1.0 mL/kg) on GD17. After weaning on P22, pups were separated by treatment group and sex and housed in groups of 2-3 in tub cages with woodchip bedding in a ventilated rack. At P60-70 (prior to the start of experiments) animals were housed individually. All experiments utilized both male and female animals from at least 2 MAM-treated (MAM) and 2 saline-treated (CTL) litters. Prior studies have found consistent behavioral deficits across both sexes in MAM animals (Perez et al. 2019; Potasiewicz et al. 2020). While animals of both sexes were used in each experiment, we did not treat sex as a variable of interest for statistical analyses. Additionally, some rats were tested in more than one behavioral assessment but were assigned only to Chronic NIC

groups in a second experiment, ensuring that all animals in Acute NIC groups were, in fact, receiving NIC for the first time. Animals had *ad libitum* access to food (LabDiet Rodent 5001) and water in the home cage throughout the duration of the experiment unless otherwise noted. Facilities were maintained on a reversed 12-hour light-dark cycle (lights off 0700), and all experimental procedures were carried out during the dark phase under dim red light illumination. All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Nicotine. Nicotine hydrogen tartrate salt (MP Pharmaceuticals) was dissolved in 0.9% sterile saline, passed through a 0.22 μM filter to ensure sterility, and stored at 4° C with minimal light exposure until experimental use. Nicotine solutions were made every 2-4 weeks. A moderate (0.3 mg/kg, s.c.) dose of NIC was selected in order to minimize adverse effects on behavior, such as lethargy, while maintaining relevance to dosing in smokers (Matta et al. 2007). Animals tested in "chronic NIC" conditions were injected with NIC (0.3 mg/kg, s.c.) daily for 12 – 24 days prior to experimental testing unless otherwise noted. Those tested in "acute NIC" conditions received daily injections of SAL (1.0 mL/kg, s.c.) daily for at least 12 days to control for effects of repeated injections in the chronic NIC groups. This acute/chronic paradigm was utilized in our PPI and NOR experiments, but could not be followed in the ASST experiment as the progressive, multi-session format of the task precluded study of acute NIC effects. Animals in the ASST experiment thus received either chronic daily NIC or chronic SAL injections for the duration of the study, beginning three days prior to the start of testing.

Prepulse inhibition of startle. Assessment of auditory PPI in MAM and CTL animals was adapted from the protocol used by Moore and colleagues (Moore et al. 2006). Thirty minutes after

injection with NIC or SAL, rats were placed into transparent Plexiglas restraint tubes inside startle chambers (San Diego Instruments, Inc., San Diego, California) and allowed to habituate to a 60 dB white noise background for five minutes. PPI was determined using 21 trials of a 105 dB startle tone (40 ms in length) preceded by prepulses 7 or 11 dB above background (12 ms in length) with an intertrial interval of 15-25 seconds. Seven trials contained the startle tone alone. Startle response (measured as arbitrary force units) was measured for each trial and percent PPI was calculated for each prepulse intensity as a percent change in response from startle-only trials. Animals in both groups were tested once after SAL injection (Pre-NIC Baseline; 24 hr abstinent for Chronic NIC animals) and again after NIC injection (Post-NIC). These trials were separated by at least 48 hrs.

Novel object recognition. Behavioral testing took place in a black Plexiglas arena (43 cm W x 43 cm L x 32 cm H) in a moderately illuminated testing room as described by Stark et al. (Stark et al. 2019). For each testing session, animals were placed in the arena with two identical objects placed in opposite corners and allowed to explore for five minutes (familiarization phase), after which they were returned to their transport cage for an inter-trial interval of 1 hour. Objects used were brown glass bottles, metallic cylinders, and nonporous ceramic figurines, and pairings of novel and familiar objects were randomized across animals. Fifteen minutes before the testing phase, animals were injected with NIC (0.3 mg/kg, s.c.) or SAL (1.0 mL/kg). During the testing phase, animals were again placed in the Plexiglas arena with one familiar object and one novel object (placement in corners was counterbalanced across animals) and again allowed to freely explore for 5 minutes. This exploration was recorded and videos were later scored for interaction time with each object by an experimenter blinded to subjects' drug conditions in order to calculate percent interaction time with novel object ([novel time/(total interaction time)] x 100).

"Interaction" was defined as touching, sniffing, or orientation of the snout toward the object with a distance of <2 cm. Sitting on top of the object was not scored as interaction. The arena and all objects were cleaned with 70% ethanol between each testing period.

Attentional set-shifting. Food restriction for attentional set-shifting (ASST) subjects began at least 24 hr prior to the first experimental session. Rats were allotted 20 grams (males) or 15 grams (females) of chow (LabDiet Rodent 5001) daily after each behavioral session to maintain approximately 80% of free-feeding weight. Injections of SAL or NIC (0.3 mg/kg, s.c.) were administered 30 minutes prior to behavioral sessions. Due to the continuous nature of testing over a number of days, an Acute NIC condition was not possible; animals were thus assigned to Chronic SAL or Chronic NIC only. Testing took place in operant chambers (30.5 cm × 24.1 cm × 21.0 cm; ENV-008CT; Med-Associates) enclosed in individual sound-attenuating cubicles. Each chamber was equipped with a feeder and receptacle on one wall and a touch screen (iPad; Apple, Inc.) exposed by two square windows on the opposite wall. Set-shifting task sessions were run through K-Limbic software and touch screen responses were recorded. The progression of session criteria is detailed in Figure 5. Correct responses in each session were reinforced by the delivery of 20 mg sucrose pellets (Bio-Serv, Flemington, NJ).

Statistical analyses. Data analyses were conducted in IBM SPSS Statistics and GraphPad Prism. Data from PPI and NOR experiments were analyzed using separate two-way repeated measures ANOVA for Acute vs. Chronic NIC animals, with Group (MAM vs. CTL) and Drug (Pre- vs. Post-NIC) as factors. Trials to criterion in each ASST phase were analyzed using two-way ANOVA with Group (MAM vs. CTL) and Drug (SAL vs. NIC) as factors. Post-hoc comparisons were made using t-tests. Individual analyses used in each experiment are described in greater detail with the results of the individual experiments.

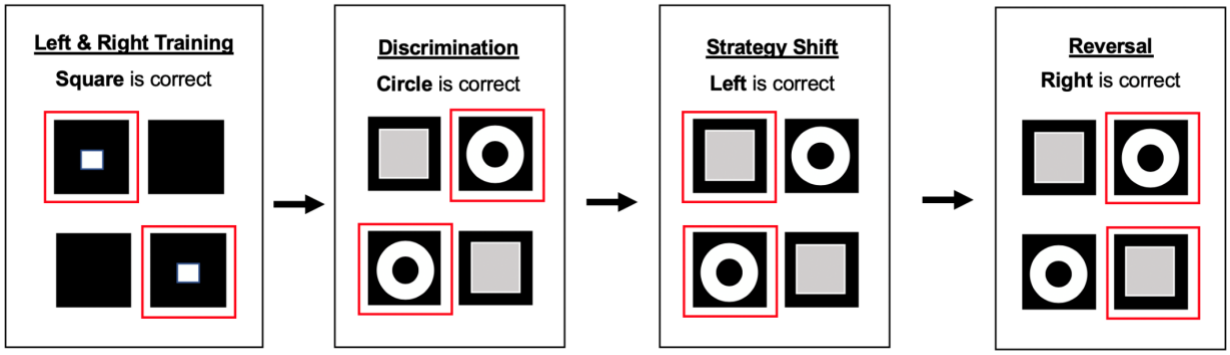

Figure 5: ASST session progression

The ASST protocol consisted of four main phases depicted above. Images in each box depict possible stimuli presentations on the two touch screens within the operant box; correct response options for each phase are outlined in red. The Left & Right Training phase required 85+ correct response trials out of 96 total stimulus presentation trials. All other phases required correct responding to 10 consecutive trials in order to proceed. Trials to reach this criterion (TTC) were compared between groups in the Strategy Shift and Reversal phases.

3.3 RESULTS

PPI deficits in MAM animals are mitigated by acute or chronic nicotine administration. Percentage of inhibition of the startle response by 7 dB and 11 dB prepulses was measured in MAM (n = 13, 4 M/9 F) and CTL (n = 13, 7 M/6 F). Although MAM animals demonstrated a PPI deficit at both 7 dB and 11 dB prepulse intensities, only the analyses from 7 dB trial data are summarized here due to high variability in MAM animals' responses at 11 dB. In the Acute NIC experiment, there was an effect of Group ($F_{1,24} = 4.69$, $p < 0.05$) and of Drug ($F_{1,23} = 18.52$, $p < 0.0005$) and a Group x Drug interaction ($F_{1,23} = 4.68$, $p < 0.05$) on % PPI (Figure 6A). Post-hoc t-tests confirmed that, at Pre-NIC Baseline, MAM animals demonstrated a significantly lower % PPI than CTL animals (-2.52% ± 9.24% vs. 27.75% ± 7.18%; $p < 0.05$). Acute NIC injection significantly increased % PPI in MAM animals ($t_{12} = 4.17$, $p < 0.005$). In the Chronic NIC experiment, there were no significant effects of Drug or Group or a Drug x Group interaction (Figure 6B). MAM (n = 12, 5 M/7 F) and CTL (n = 7, 4 M/3 F) animals did not differ in % PPI at Pre-NIC Baseline (24 hr abstinent from NIC) (26.60% ± 8.82% vs. 39.58% ± 8.89%; $p = 0.35$). NIC administration further increased % PPI from Pre-NIC Baseline in MAM animals (46.20% ± 5.67%; $p < 0.05$).

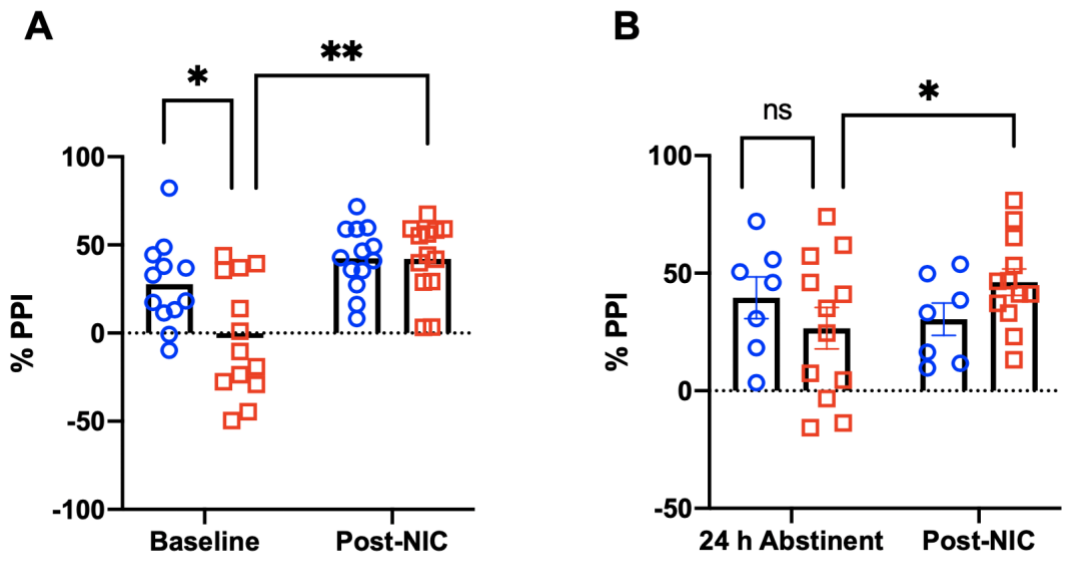

Figure 6: Acute and chronic NIC normalizes prepulse inhibition of startle in MAM animals

Average percent inhibition of startle response in prepulse trials (%PPI). (A) CTL animals demonstrated significantly higher % PPI at Baseline than MAM animals. Acute NIC normalized this difference, significantly increasing % PPI in MAM subjects. (B) Chronically NIC-treated CTL and MAM animals did not differ in % PPI at a 24-hr abstinent baseline, and NIC injection further increased % PPI in MAM animals. *CTL rats, blue circles; MAM rats, red squares; * $p < 0.05$; ** $p < 0.01$.*

Acute and chronic nicotine normalizes novel object recognition in MAM animals. A two-way repeated measures ANOVA of percent interaction time with novel object in drug-naïve MAM (n = 9, 4 M/5 F) and CTL animals (n = 8, 4 M/4 F) before and after acute NIC revealed a Group x Drug interaction ($F_{1,15} = 12.05$, $p < 0.005$; Figure 7A). At Pre-NIC Baseline, MAM animals spent a significantly lower percentage of time interacting with the novel object than CTL animals (62.70% ± 4.03% vs. 75.02% ± 1.82%; $p < 0.05$). However, MAM animals spent significantly more time interacting with the novel object after NIC administration than at Pre-NIC Baseline (74.07% ± 2.46%; $p < 0.05$) and were not different from CTL animals Post-NIC (68.41% ± 4.04%; $p = 0.24$). In the Chronic NIC experiment, there was no Group x Drug interaction and no effect of Group or Drug on percent interaction time with novel object (Figure 7B). MAM (n = 7, 3 M/4 F) and CTL (n = 7, 4 M/3 F) animals did not differ when tested after 24 hrs abstinent from NIC (Pre-NIC Baseline; $p = 0.19$) or after NIC injection (Post-NIC; $p = 0.65$). Total object interaction times in both experiments were compared for MAM and CTL animals to confirm comparable exploration between groups. There were no significant effects of Group ($p = 0.93$) or Drug ($p = 0.36$) and no Group x Drug interaction ($p = 0.66$) on total object interaction time in the Acute NIC experiment. In the Chronic NIC experiment, there was no effect of Group ($p = 0.56$) and no Group x Drug interaction ($p = 0.76$), but there was a significant effect of Drug ($F_{1,12} = 13.51$, $p < 0.005$). Total object interaction time was significantly reduced in both groups after NIC administration, but they did not differ at either timepoint.

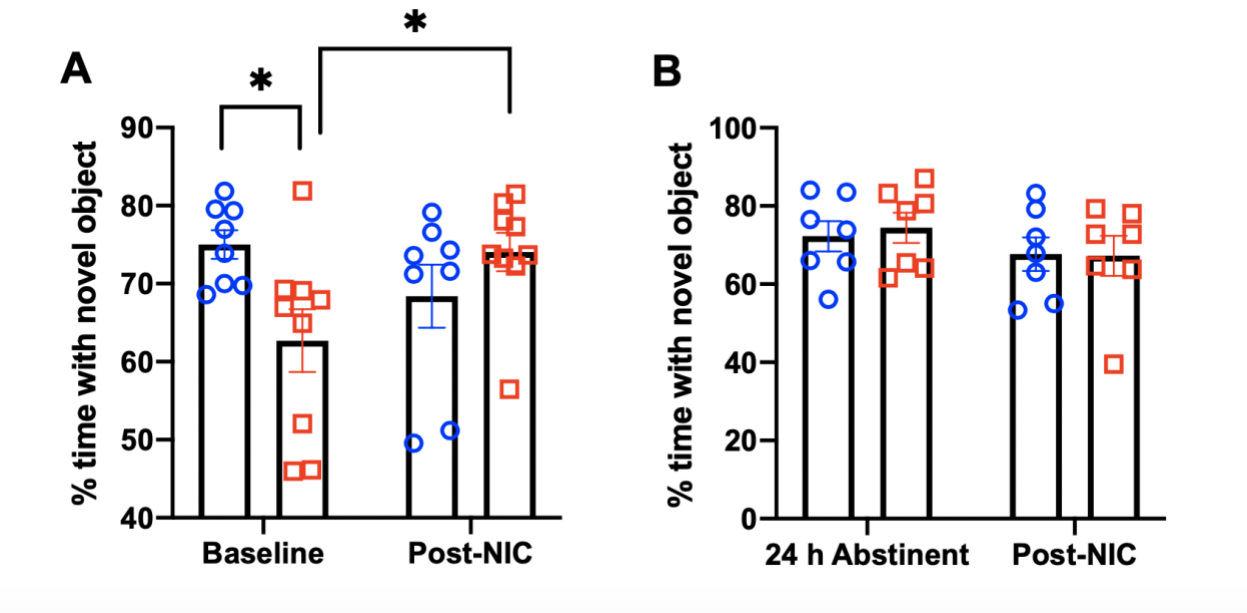

Figure 7: Acute and chronic NIC normalizes novel object recognition in MAM animals

Percentage of total interaction time spent with novel object during NOR task. (A) At baseline, CTL animals spent a significantly greater proportion of time interacting with the novel object than MAM animals. Acute NIC administration significantly increased MAM animals' interaction time with the novel object to a level comparable to CTL animals. (B) Chronically NIC-treated MAM and CTL animals did not differ in percent interaction time with the novel object at a 24-hr abstinent baseline or after acute NIC administration. *CTL rats, blue circles; MAM rats, red squares; * $p < 0.05$.*

Nicotine improves strategy shifting in MAM animals but does not affect reversal learning. A two-way ANOVA of trials to criterion in the Strategy Shift (Figure 8A) and Reversal (Figure 8B) phases revealed no effect of Drug or Group and no Drug x Group interaction. Despite evidence of impaired performance in MAM animals on reversal and set shift phases in previous versions of the ASST (Gastambide et al. 2012), SAL-treated MAM (n = 6, 3 M/3 F) and CTL (n = 6, 4 M/2 F) animals did not differ in trials to criterion (TTC) in equivalent phases of this protocol. Interestingly, however, NIC-treated MAM animals required significantly fewer TTC (104.25 ± 13.89) in the Strategy Shift phase than both SAL-treated MAM animals (148.33 ± 13.67; $p < 0.05$) and NIC-treated CTL animals (157.44 ± 14.27; $p < 0.05$). These results suggest that MAM animals do not display measurable deficits on this version of the ASST. However, NIC administration does selectively improve MAM animals' performance in the Strategy Shift phase.

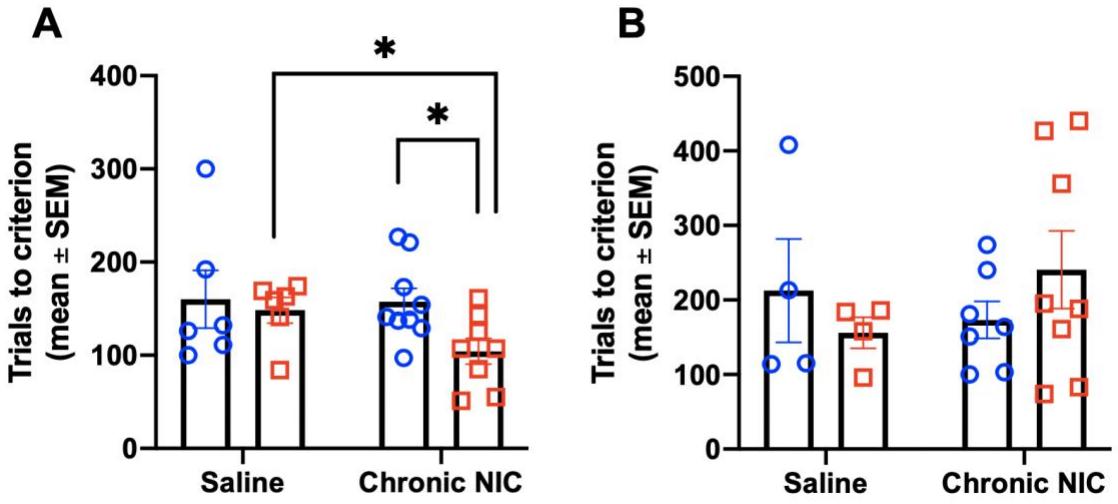

Figure 8: Nicotine improves MAM animals' performance in the strategy shift phase of the ASST

Mean trials required to meet progression criteria in the ASST. (A) Saline-treated MAM and CTL animals did not differ in performance on the Strategy Shift phase of the ASST. MAM animals treated with NIC throughout the experiment required significantly fewer trials to reach criterion than saline-treated MAM animals and NIC-treated CTL animals. (B) CTL and MAM animals did not differ in performance during the Reversal phase of the ASST, regardless of drug treatment. *CTL rats, blue circles; MAM rats, red squares; * $p < 0.05$.*

3.4 DISCUSSION

Our work aims to explore mechanisms underlying increased smoking in SCZ using the MAM rodent model of the disease. Previously, we have demonstrated that NIC reinforcement is not increased in these animals, failing to support the hypothesis that SCZ pathophysiology confers increased NIC reward, driving continued NIC use. We therefore aimed to evaluate the self-medication hypothesis, which proposes that NIC normalizes some cognitive symptoms of SCZ, in this study. These experiments sought to characterize the effects of acute and chronic NIC treatment on measures of sensory gating and cognitive deficits in a well-established rodent model of SCZ. Our findings suggest that both acute and chronic administration of NIC can normalize auditory gating and short-term memory deficits in the MAM rodent model of SCZ, and that chronic administration may improve cognitive flexibility in these animals. To our knowledge, this study is the first to examine the effects of NIC on these behaviors in the MAM model and is unique in its comparison of acute and chronic effects of the drug.

Our study utilized auditory PPI as a measure of sensory gating in MAM and CTL rats. This assessment is a consistently demonstrated analog of sensory gating dysfunction in SCZ that is known to be mediated by nicotinic receptors and is reliably reproduced by the MAM model (Pinnock et al. 2015; Swerdlow et al. 1999; Ewing and Grace 2013). Our experiments confirmed a reduction in baseline PPI in MAM rats relative to CTL. Acute injection of NIC significantly increased PPI in MAM animals, fully normalizing deficits in this measure. This replicates clinical findings of NIC enhancement of PPI in CTL nonsmokers and parallels NIC normalization of P50 sensory gating deficits in nonsmoking first-degree SCZ relatives (Kumari et al. 1997; Adler et al. 1993). In order to model how chronic NIC intake may alter baseline and post-NIC PPI in smokers, we also tested PPI in MAM and CTL animals treated for 12+ days with NIC at a 24-hr abstinent

baseline and after acute NIC injection. We found that, at 24 hrs abstinent from NIC, MAM and CTL animals did not differ in PPI. While these were two separate experiments and thus not compared statistically, we did observe that these baseline levels of PPI in the chronically-treated animals were similar to the baseline PPI observed in saline-treated CTL rats, suggesting that this lack of difference likely reflects an enhancement of PPI in MAM animals rather than a reduction in CTL animals. Additionally, acute injection of NIC further enhanced PPI in chronically-treated MAM animals, suggesting that these animals did not develop tolerance to the PPI-enhancing effects of NIC. These findings also reflect clinical observations that acute smoking improves PPI in both nonpsychiatric and SCZ smokers (Hong et al. 2008; Kumari et al. 2001). However, unlike rats measured at 24 hrs abstinent from NIC, SCZ smokers have demonstrated exacerbated PPI deficits after overnight abstinence, which are also normalized by smoking (George et al. 2006). Nonetheless, our findings do suggest that acute NIC normalizes PPI deficits in MAM animals, and that this effect persists even after chronic NIC administration, potentially supporting the hypothesis that continued smoking in SCZ may be motivated by symptom relief. Importantly, these findings also validate this model as a useful tool in future studies of the effects of NIC and nicotinic drugs on sensory gating deficits.

Our experiments aimed to assess the effects of NIC on episodic memory and novelty discrimination in the MAM model using the NOR task, a metric in which MAM animals consistently display deficits (Stark et al. 2019; Flagstad et al. 2005). While studies of memory impairments in SCZ patients are relatively complex and multidimensional, this preclinical assessment still holds relevance to clinical observations of impaired novelty discrimination in SCZ (McGuire et al. 2013). A 4-hr application of transdermal NIC prior to testing significantly improved performance on a novelty detection task in SCZ nonsmokers, demonstrating that acute

NIC treatment may mitigate episodic memory deficits in SCZ subjects (Jubelt et al. 2008). However, other findings suggest that NIC may only improve recognition memory performance in SCZ smokers, but not nonsmokers (Myers et al. 2004). Our experimental design allowed for assessment of acute NIC effects in modeled "nonsmoking" vs. "smoking" conditions by testing animals repeatedly administered saline or NIC. In animals receiving repeated injections of saline, we observed a significant reduction in novel object interaction time in MAM animals relative to CTLs, and that this difference was normalized by an acute injection of NIC. After chronic treatment with NIC, MAM and CTL animals did not differ in novel object interaction time at a 24-hr abstinence baseline or when tested after acute NIC injection. In these experiments, acute NIC injections took place prior to the testing trial, suggesting that effects observed in testing may reflect an enhancement of the retrieval of object-associated memory. However, in studies of control animals, $\alpha 7$ and $\alpha 4\beta 2$ nAChR agonists have been demonstrated to enhance novel object recognition when administered before the acquisition or the testing trial, suggesting that stimulation of these receptors may play a role in both the formation and retrieval of memories (McLean et al. 2016). Future experiments could provide insight into specific elements of memory dysfunction in the MAM model by assessing how the timing of injections may impact the observed effects of NIC on NOR.

We did not observe a difference in performance of the ASST, an assessment of attention and cognitive flexibility, between CTL and MAM animals receiving saline injections. CTL and MAM animals required a similar number of trials to reach criterion in both the Strategy Shift and Reversal phases, despite evidence that MAM animals require more trials in these phases in other versions of the ASST (Gomes et al. 2014; Potasiewicz et al. 2020). The touch-screen format of this version of the ASST had not yet been validated in MAM animals and, based on these findings,

may have critical differences from prior versions. Indeed, the classic rodent ASST is a more multisensory assessment, as categorical shifts are made between different dimensions (i.e. textures and scents of digging media) in order to locate rewards (Heisler et al. 2015). This version of the task relies on different stimulus pairs within the visual dimension only (i.e. presentation of circle vs. square, left vs. right), making shifts in attention more strategy-based within a single dimension. Despite evidence of impaired intradimensional shifting in MAM animals in prior studies, the touch-screen task used in our study did not reveal a difference in performance between MAM and CTL animals (Gomes et al. 2014). In the traditional digging ASST format, salient stimuli of another sensory dimension, such as texture, are present in the testing milieu even when discrimination rule shifts are occurring within a single dimension, such as odor. This provides a greater field of perceptual features within which animals must selectively attend (Birrell and Brown 2000). It is thus possible that this protocol does not capture the multisensory complexity of prior versions, which may be necessary to detect a significant behavioral difference. Interestingly, we also found that NIC-treated MAM animals required significantly fewer trials to reach criterion in the Strategy Shift phase of our task than SAL-treated animals or NIC-treated CTL animals. This selective improvement observed in MAM animals was surprising, as prior studies of the ASST have demonstrated that repeated NIC treatment does improve performance of control rats in both the intradimensional and extradimensional shift phases (Allison and Shoaib 2013). It is thus possible that the mechanism by which NIC selectively improves performance in MAM animals may be specific to the model endophenotype, a possibility that can be explored in future experiments.

Evaluation of the effects of acute NIC in both drug-naïve and chronically-treated animals was a critical and unique element of this study. In doing so, we aimed to model both baseline

performance and NIC effects in “nonsmokers” versus “smokers” in order to determine how repeated NIC exposure may change later responses within subjects. Particularly, we were interested in determining how chronic NIC treatment may impact behavior in the absence of NIC, which we measured using a 24-hr abstinent baseline. Despite evidence of exacerbated cognitive and sensory gating deficits in briefly- abstinent SCZ smokers due to withdrawal symptoms, we did not find differences in performance at the 24-hr abstinent baseline of chronically NIC-treated MAM and CTL animals (Harris et al. 2004; George et al. 2002). This parallels the results of a study by Boggs and colleagues (2018), which demonstrated that both brief and prolonged smoking abstinence did not affect performance of SCZ subjects on a test battery assessing multiple cognitive domains. Additionally, our results suggested that, in measures of PPI and NOR, the effects of acute NIC administration do differ between drug-naïve and chronically-treated animals. While chronic administration does appear to normalize baseline behavior, additional responses to acute NIC treatment may vary by measure, as there was no further effect of NIC on NOR in chronically-treated MAM and CTL animals. There are important limitations to our study as well. The use of a single, experimenter-administered daily dose in our chronic treatment protocol does not necessarily reflect human smoking, as smokers are able to self-regulate NIC intake by choosing when and how many cigarettes to smoke. Though MAM and CTL animals do not differ in NIC self-administration and thus experience similar reinforcing effects of NIC, it is likely that the cognitive effects we observed may differ across different dosing paradigms (Weeks et al. 2020).Additionally, as our acute and chronic NIC treatment groups were studied in two separate experiments, we cannot statistically compare these groups and can only make basic overall comparisons. Our findings demonstrated that baseline PPI and NOR deficits in MAM animals are normalized by acute NIC and that chronically treated MAM and CTL animals do not differ when

tested at acute abstinence or after NIC. However, we cannot statistically determine that our observations in chronically-treated animals reflect a sustained normalization in MAM animals rather than a reduction in CTL animals' performance. Future experiments could utilize different treatment schedules within a single experiment in order to make these comparisons possible. Overall, these findings suggest that NIC administration can have persistent enhancement effects on sensory gating and episodic memory in the MAM model, though further study is needed to draw conclusions on how this motivate continued NIC use in humans.

The results of these experiments suggest that administration of a moderate dose of NIC can enhance measures of sensory gating, recognition memory, and cognitive flexibility in the MAM model of SCZ. These findings lend modest support to a self-medication hypothesis behind increased smoking in SCZ by demonstrating that NIC may alleviate deficits resulting from a SCZ-parallel endophenotype. While these experiments illustrate the potential utility of the MAM model in further exploring nicotinic mechanisms of cognitive enhancement in SCZ, they cannot provide direct insight regarding how these cognitive effects of NIC may motivate patients to smoke more. When surveyed, SCZ smokers were not more likely than controls to cite cognitive enhancement as a motivator for smoking, though relief of negative affect was more consistently reported among patients (Galazyn et al. 2010; Forchuk et al. 2002). Additional studies suggest that healthy controls, but not SCZ subjects, self-report increases in concentration after acute NIC delivery (Hahn et al. 2013) This raises the possibility that, despite many instances of cognition-enhancing effects of NIC in clinical studies of SCZ smokers, these effects may not necessarily improve daily functioning in a noticeable way. A self-medication hypothesis could thus encompass a general sense of "feeling better", rather than discrete effects on cognitive performance, as a motivator for increased smoking in SCZ. Nonetheless, these findings offer valuable evidence linking specific

elements of SCZ neurophysiology modeled by GD17 MAM with relevant behavioral measures affected by NIC. This study adds to a body of evidence suggesting the potential of nicotinic agonists in improving cognitive dysfunction in SCZ. Further knowledge in this area may also inform more targeted approaches in reducing smoking among SCZ patients by highlighting distinct areas of focus for improving cessation and reducing cognitive symptoms of withdrawal.

4.0 NICOTINE NORMALIZES ABNORMALITIES IN DOPAMINERGIC AND HIPPOCAMPAL CELL ACTIVITY IN THE MAM RODENT MODEL OF SCHIZOPHRENIA

4.1 INTRODUCTION

Despite considerable progress in reducing tobacco use worldwide, disparities remain in this public health effort. An estimated 60% of individuals with schizophrenia (SCZ) are current smokers, representing roughly three times the proportion of smokers in the general population (Dickerson et al. 2018). This elevated rate of smoking among SCZ patients has long been hypothesized as an attempt to "self-medicate" negative and cognitive symptoms of the disease through intake of nicotine (NIC), the primary reinforcing component of tobacco (Kumari and Postma 2005). While direct experimental tests of whether effects of NIC on certain SCZ symptoms serve as a motivator of smoking have yielded mixed results, evidence does suggest consistent modulatory effects of nicotinic agonists on certain deficits in SCZ (AhnAllen 2012; Tregellas and Wylie 2019). A better understanding of motivators behind smoking and of the neurophysiological effects of NIC that may be unique to SCZ smokers is critical to reducing tobacco use in this population. Additionally, more focused study of nicotinic modulation of system dysfunction in SCZ could inform the development of effective pharmacotherapies.

The majority of studies assessing the idea that smoking serves as a form of "self-medication" in SCZ have focused on measurable behavioral effects of NIC. While findings are mixed, a number of studies suggest that NIC intake can acutely improve sensorimotor gating deficits, negative symptom severity, and some measures of cognitive function, particularly

attention, in patients, though this effect may vary by smoking status (Postma et al. 2006; Smith et al. 2002; Harris et al. 2004). Clinical studies of the neurophysiological effects of NIC underlying these behavioral changes are challenging due to ethical limitations, which highlights the utility of animal models of SCZ. While SCZ is a distinctly human disease with no precisely determined etiology, animal models capturing known neurophysiological disruptions observed in the affected brain are a versatile tool in mechanistic studies. Models developed through pharmacological manipulation or neurodevelopmental insult, for example, display systems-level neuronal dysfunction that parallel many observations in SCZ. Disruptions in functional coupling between prefrontal regions and its inputs are thought to contribute to negative symptoms and cognitive impairments and may be modulated by nicotinic activation (Pratt et al. 2008). For example, in the rodent chronic ketamine model of SCZ, reductions in GABAergic transmission in orbitofrontal cortex are linked with deficits in multisensory integration and normalized by activation of α4β2 nicotinic acetylcholine receptors (nAChRs) in this region (Cloke et al. 2016). NIC administration has also been demonstrated to reverse hypofrontality observed in a genetic model of the disease by increasing inhibitory drive of interneurons in prefrontal cortex (PFC) (Koukouli et al. 2017)

Importantly, a reduction in parvalbumin-positive (PV) inhibitory interneurons is observed throughout mPFC and ventral subiculum in several animal models, as well as in post-mortem tissue of SCZ patients (Lodge et al. 2009; Lewis and Moghaddam 2006). Evidence suggests that both the expression and function of PV cells, which are critical in regulating excitatory/inhibitory balance, are associated with nAChR function. In the hippocampus, α7 nAChRs are highly expressed on PV interneurons and are integral in regulating inhibitory synapses (Kawai et al. 2002). While non-α7 nAChRs play a greater role in regulating inhibitory balance in PFC, functional cortical α7 nAChRs are necessary in development for expression of PV interneurons in

this region (Lin et al. 2014; Arroyo et al. 2012). These observations elucidate a number of possible avenues by which NIC may affect neurophysiological function, both in SCZ and in animal models that effectively SCZ pathology.

The methylazoxymethanol acetate (MAM) model is a well-validated neurodevelopmental disruption model of SCZ highlighted by temporal lobe dysfunction and resulting uncoordinated activity of ventral hippocampus (vHipp) and its downstream projection regions, including ventral tegmental area (VTA) and medial PFC (mPFC)(Esmaeili and Grace 2013). Reduced expression of PV interneurons is also observed in this model, and recent evidence suggests that α7 nAChR dysfunction may also be recapitulated and have key effects on system-wide neurophysiological disruptions (Lodge et al. 2009). One study demonstrated that α7 nAChR agonist administration, either systemically or directly into vHipp, normalized elevated dopamine (DA) population activity observed in MAM animals but had no effect on controls (Neves and Grace 2018). This finding has potential implications for nicotinic effects on psychosis, which is largely thought to be a result of DA hyperactivity in SCZ, as well as greater systemic-wide effects. The lack of an effect in controls suggests that this modulatory effect may be unique to the inhibitory dysfunction observed in the MAM model. Additionally, the normalization of DA activity through vHipp implies a potential mechanism for nicotinic modulation of vHipp projections to other regions, as well as normalization of related behaviors such as sensorimotor gating deficits (Kohlhaas et al. 2015).

The present experiments thus aim to address two broad questions stemming from these findings. Firstly, while the reported α7-mediated reduction in VTA DA hyperactivity was demonstrated to occur via action in vHipp, direct effects on neuronal activity in this region were not measured (Neves and Grace 2018). The effect of NIC on neuronal activity in vHipp and its downstream afferent targets remains unclear, as α7 nAChRs have a low affinity for NIC (Tregellas

and Wylie 2019). Additionally, prior studies did not assess the effects of a single dose versus multiple doses of NIC on neuronal activity, which bears relevance to human smokers and may be altered by changes in receptor expression or function. Therefore, the goals of this study were to examine the effects of both acute and chronic NIC administration on VTA DA activity, as well as activity of putative pyramidal cells in vHipp, in MAM and CTL animals. We hypothesized that NIC administration would reduce the elevated VTA DA population activity and vHipp firing rate observed in drug-naïve MAM animals, normalizing their activity to levels observed in CTL animals. We also hypothesized that this acute effect of NIC administration on VTA DA and vHipp activity would persist after chronic NIC treatment, but also sought to determine how baseline levels of activity may be altered in an acute state of abstinence. In order to explore these hypotheses, we conducted in vivo extracellular recordings of cells from VTA or vHipp in either drug-naïve or chronically NIC-treated MAM and CTL animals. We found that NIC administration, both acute and chronic, normalizes elevations in VTA DA and vHipp activity in the MAM model of SCZ.

4.2 MATERIALS AND METHODS

Animals. All experiments utilized Sprague-Dawley rats aged 2 – 6 months at the start of experimental sessions. Rats were born in-house to timed pregnant dams (Envigo) injected intraperitoneally with saline (CTL; 1.0 mL/kg) or methylazoxymethanol acetate (MAM; 25.0 mg/kg, 1.0 mL/kg in saline) on GD17 (Lodge 2013). After weaning on P22, pups were separated by treatment group and sex and housed in groups of 2-3 in tub cages with woodchip bedding in a ventilated rack. At P60-70 (prior to the start of experiments) animals were housed individually. All experiments utilized both male and female animals from 2-3 MAM-treated (MAM) and 2-3

saline-treated (CTL) litters. Animals had *ad libitum* access to food and water in the home cage throughout the duration of the experiment. Facilities were maintained on a reversed 12-hour light-dark cycle (lights off 0700), and all recordings were performed under white light. All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs. Chloral hydrate (Sigma-Aldrich) was dissolved in 0.9% sterile saline for intraperitoneal injection and stored at 4° C with minimal light exposure. Nicotine hydrogen tartrate salt (MP Pharmaceuticals) was dissolved in 0.9% sterile saline, passed through a 0.22 μM filter to ensure sterility, and stored at 4° C with minimal light exposure until experimental use. Nicotine solutions were made every 2-4 weeks. Animals in Chronic NIC experiments were injected with NIC (0.3 mg/kg, s.c.) daily for at least 12 days prior to recordings. Those tested in Acute NIC experiments received daily injections of SAL (1.0 mL/kg, s.c.) daily for at least 12 days to control for effects of repeated injections. Acute NIC infusions (30 μg/kg, i.v.) during electrophysiological recording sessions were delivered through a catheter inserted into the right jugular vein.

Surgical preparation. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p., supplemented as necessary) and an infusion catheter was surgically placed in the right jugular vein. Rats were then secured into a stereotaxic apparatus and a core body temperature of 37° C was maintained using a thermostatically-regulated heating pad. The skull was then surgically exposed and the recording site prepared.

VTA DA neuron extracellular recordings. VTA DA neuron activity was measured as described previously (Neves and Grace 2018). Single glass microelectrodes filled with 2% Chicago Sky Blue in 2M NaCl were lowered into the ventral tegmental area (AP + 5.3, ML – 0.6 relative to bregma) using a hydraulic micropositioner. Spontaneously active neurons were

measured between -6.5 and -9.0 mm DV using a 30 kHz highpass filter and 16 kHz lowpass. Putative DA neurons meeting electrophysiological criteria of location, waveform, and firing rate were isolated and recorded for at least 60 s (Ungless and Grace 2012). Baseline population activity was determined by making 3-4 vertical passes through VTA in a random arrangement moving laterally and caudally, separated by 200 μm each. Animals were then infused with NIC (30 μg/kg, i.v.) and 3-4 more tracks were recorded to determine effects of systemic NIC on DA neuron population activity, as well as individual cells' firing rates (Hz) and percentage of spikes in bursts.

vHipp extracellular recordings. Extracellular recordings in vHipp were performed as described previously (Lodge and Grace 2007). Single glass microelectrodes filled with 2% Chicago Sky Blue in 2M NaCl were lowered into the ventral hippocampus (AP + 6.0, ML – 4.5 relative to bregma) to a depth of – 6.0 to – 8.0 mm ventral of the brain surface. Spontaneously active neurons throughout the region were recorded for at least 120 s each in a series of tracks moving laterally and caudally from the first, separated by 200 μm each. As described for DA neuron recordings, 2 - 4 vertical passes were made for baseline recordings and 2 – 4 more were made following acute infusion of NIC. Putative pyramidal cells were identified as those with spikes in a biphasic waveform, a spike width > 2.0 ms, and a firing rate < 2 Hz.

Histology. At the conclusion of each electrophysiological recording, rats were sacrificed with an overdose of chloral hydrate (addl. 400 mg/kg, i.p.). The recording site was marked by an electrophoretic injection of Chicago Sky blue (- 20 μA for 20-30 minutes). Animals were then decapitated and brains were fixed in 8% paraformaldehyde in 0.2 M PBS and cryoprotected in 25% sucrose in 0.2 M PBS. Sixty-micrometer sections were taken throughout VTA or vHipp and stained with 95% Neutral Red and 5% Cresyl Violet in order to verify electrode placement. Due

to laboratory closures caused by the COVID-19 pandemic, brains from a subset of animals in the Acute NIC vHipp recording experiment could not be processed.

Data analysis. Data collected during electrophysiological recordings were analyzed using LabChart and NeuroExplorer software to determine firing rate and, in DA neuron recordings, percentage of spikes occurring in bursts. DA population activity, defined as the average number of spontaneously active neurons encountered per electrode track, was calculated for each animal at baseline and after NIC infusion. Values are reported as mean ± SEM unless otherwise stated. Statistical analyses were performed using GraphPad Prism 8. Analyses of firing rate, bursting, and population activity within each experiment were conducted using a two-way mixed ANOVA, with Group (MAM vs. CTL) as between-subjects factors and Drug (Baseline vs. Post-NIC) as within-subjects factors when possible, followed by post-hoc tests ($\alpha = 0.05$; reported p values are adjusted for multiple comparisons using Bonferroni correction).

4.3 RESULTS

Acute NIC treatment normalizes DA population activity in MAM animals. Analysis of population activity in drug-naïve MAM (n = 7 rats; 3 M/4 F) and CTL animals (n = 5 rats; 4 M/1 F) at baseline and after NIC treatment revealed a significant effect of Drug ($F_{1,10} = 9.0$, $p < 0.05$) and a significant Group x Drug interaction ($F_{1,10} = 4.8$, $p = 0.052$). Post-hoc tests indicated that MAM animals showed significantly elevated DA population activity at baseline (1.01 ± 0.01 cells/track) relative to CTL animals (0.66 ± 0.07 cells/track, $p < 0.05$). However, as hypothesized, population activity in MAM animals was significantly reduced after NIC administration (0.44 ± 0.15 cells/track, $p < 0.01$; Figure 9A). There were no significant effects of Group or Drug on average firing rate or on bursting (Figure 9B,C.)

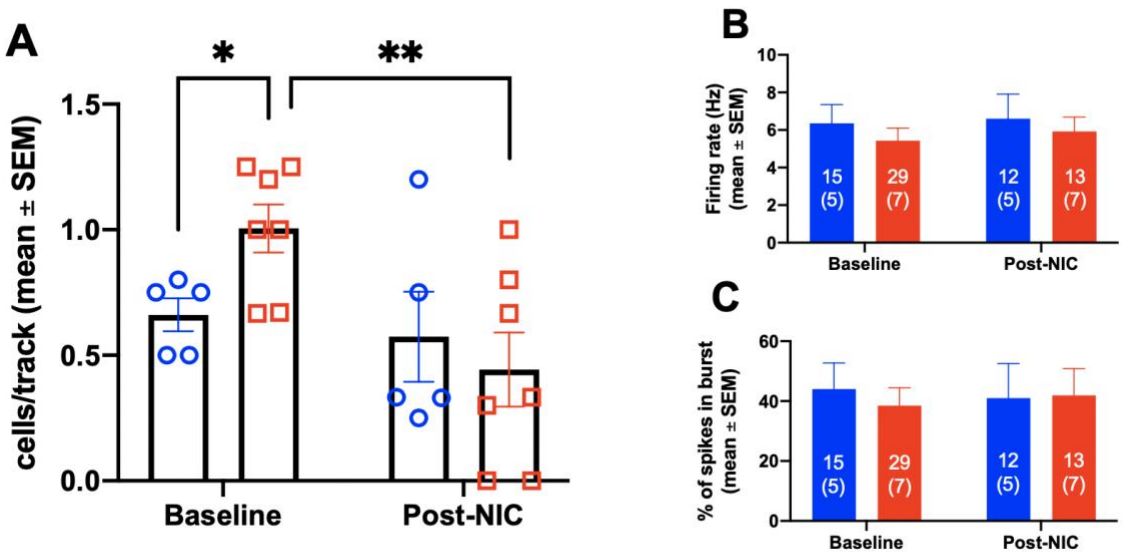

Figure 9: Acute NIC normalizes elevated DA population activity in MAM animals

Electrophysiological data collected from spontaneously active neurons in VTA. (A) At Baseline, population activity of VTA DA neurons was significantly higher in MAM than CTL animals. Acute systemic NIC infusion normalized this elevated activity, significantly lowering the number of cells encountered per track relative to Baseline. (B, C) MAM and CTL animals did not differ in mean firing rate or percentage of spikes in burst at Baseline or Post-NIC. *CTL rats, blue circles/bars; MAM rats, red squares/bars; * $p < 0.05$; ** $p < 0.01$; Bar labels indicate number of cells analyzed (number of animals).*

DA population activity is comparable in MAM and CTL animals after chronic NIC. MAM and CTL rats (n = 6 per group; 4 M/2 F) were injected with NIC (0.3 mg/kg, s.c.) daily for at least 12 days. When measured 24 hours after their last injection (Baseline) and then after a single infusion of NIC (Post-NIC), there was no effect of Drug ($p = 0.18$) or Group ($p = 0.29$) and no Drug x Group interaction ($p = 0.56$) on VTA DA population activity in MAM and CTL rats (Figure 10A). Similarly, there were no effects on average firing rate or on bursting in these animals (Figure 10B,C).

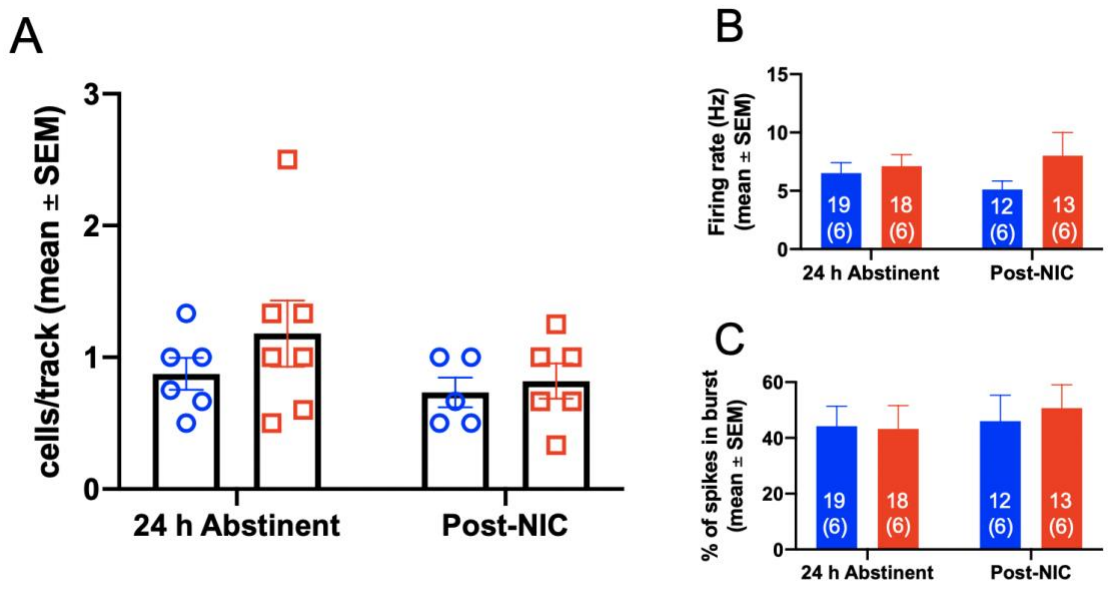

Figure 10: VTA DA neuron activity is comparable in CTL and MAM animals after chronic NIC

Electrophysiological data collected from spontaneously active neurons in VTA. (A) CTL and MAM animals chronically treated with NIC demonstrated comparable VTA DA population activity at a 24 h Abstinent baseline and after acute NIC infusion. (B, C) MAM and CTL animals did not differ in mean firing rate or percentage of spikes in burst at Baseline or Post-NIC. *CTL rats, blue circles/bars; MAM rats, red squares/bars; Bar labels indicate number of cells analyzed (number of animals).*

Acute NIC significantly reduces elevated vHipp firing rates in MAM animals. Analysis of average firing rates of putative pyramidal cells in vHipp of MAM (n = 8 rats; 5 M/3 F) and CTL (n = 8 rats; 5 M/3 F) animals revealed a significant effect of Group ($F_{1,28} = 5.96$, $p < 0.05$, Figure 11A) but no effect of Drug ($p = 0.27$) and no Group x Drug interaction ($p = 0.17$). A Student's t-test of average firing rates in drug-naïve animals supported the *a priori* hypothesis that firing rates in MAM animals (1.23 ± 0.1 Hz) were significantly higher than those in CTL animals (0.84 ± 0.11 Hz) measured at Baseline ($t_{14} = 2.69$, $p < 0.05$). Additionally, average Post-NIC firing rates (0.98 ± 0.11 Hz) in MAM animals were significantly lower than those measured at Baseline ($t_7 = 2.45$, $p < 0.05$) and not significantly different from Baseline firing rates in CTL animals ($p = 0.38$).

Average vHipp neuronal firing rates do not differ in MAM and CTL animals after chronic NIC treatment. As in the DA recording experiment, MAM (n = 7 rats; 4 M/3 F) and CTL rats (n = 7 rats; 3 M/4 F) were injected with NIC (0.3 mg/kg, s.c.) daily for at least 12 days. A two-way ANOVA of averaged neuronal firing rates in each animal revealed no significant effects of Group ($p = 0.53$) or Drug ($p = 0.43$) and no Group x Drug interaction ($p = 0.27$; Figure 11B). As hypothesized, mean firing rates of cells recorded from vHipp in chronically treated MAM (0.88 ± 0.2 Hz; n = 17 cells/7 animals) and CTL animals (0.82 ± 0.08 Hz; n = 23 cells/7 animals) did not differ after acute NIC infusion ($t_{11} = 0.31$, $p = 0.76$).

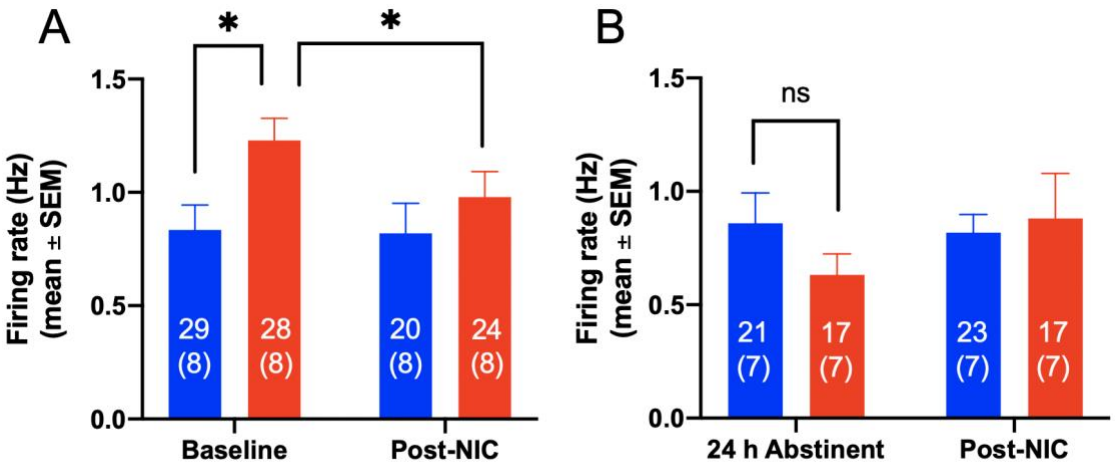

Figure 11: Acute and chronic NIC normalizes elevated firing rates of vHipp neurons

(A) Mean firing rates of spontaneously active neurons encountered in vHipp were significantly higher in NIC-naïve MAM animals than CTL at Baseline. However, acute systemic infusion of NIC significantly decreased mean firing rates in MAM animals, normalizing this elevation. (B) vHipp neuronal firing rates measured in chronically NIC-treated MAM and CTL animals did not differ when measured at a 24 h Abstinent baseline or after acute NIC infusion. *CTL rats, blue bars; MAM rats, red bars; * $p < 0.05$; Bar labels indicate number of cells analyzed (number of animals).*

4.4 DISCUSSION

These experiments aimed to examine the effects of acute and chronic NIC on neurophysiological perturbations in the MAM rodent model of SCZ. High rates of smoking among SCZ patients are often hypothesized to be a form of "self-medication", driven by NIC-induced relief of some symptoms of the illness. While evidence does suggest some mixed positive effects of NIC on certain cognitive and behavioral symptoms, little is known about the impact of NIC intake on neurophysiological mechanisms underlying these symptoms (Valentine and Sofuoglu 2018). Ventral hippocampal hyperactivity driving elevated VTA DA neuron activity is a mechanistic hallmark of the MAM model, and the disruptions in both VTA and vHipp activity in model animals parallel those observed in many neuroimaging studies of patients (Modinos et al. 2015). Based on prior evidence that NIC can improve some behavioral deficits and that systemic α7 nAChR agonists can normalize elevated DA population activity in MAM animals via action in vHipp, our experiments sought to explore a potential link between these observations (Neves and Grace 2018). We conducted *in vivo* electrophysiological recordings of VTA DA and vHipp pyramidal cell activity to test the hypothesis that acute NIC can normalize hyperactivity in both regions. Our findings demonstrate that a single, behaviorally-relevant systemic dose of NIC normalizes elevated VTA DA population activity observed in MAM animals and that this effect persists after chronic NIC treatment, even in acute abstinence. Acute NIC administration also significantly reduced elevated vHipp neuron firing rates observed in MAM animals, and differences between MAM and CTL animals remain mitigated after chronic NIC treatment. These results confirm the hypothesis that NIC can mitigate DA population hyperactivity in the MAM model and may support a direct mechanism via reduction of vHipp hyperactivity.

These experiments aimed to model the neurophysiological impact of NIC administration in smoking versus nonsmoking patients captured in human studies by examining the effects of acute NIC doses at both a drug-naïve baseline and at a 24-hour abstinent baseline in chronically treated animals. This distinction is particularly critical, as findings on the cognitive and behavioral effects of NIC in SCZ patients can vary widely by smoking status. Indeed, some cognitive deficits in SCZ smokers may be further exacerbated by acute NIC withdrawal, while positive effects of NIC on performance may be most robust in nonsmokers (AhnAllen et al. 2008; Jubelt et al. 2008). Prior studies in the MAM model have demonstrated positive effects of acute NIC and nAChR agonists on sensory gating deficits and DA hyperactivity, but our study is the first to examine comprehensively the effects of both acute and chronic NIC admin on VTA and vHipp neuronal activity in this model (Kohlhaas et al. 2015; Neves and Grace 2018). Because drug-naïve and chronically-treated animals were studied in separate experiments at separate times, we cannot make direct statistical comparisons between the results of these experiments. It is thus challenging to interpret at face value the finding that DA population activity measured at a 24-hr Abstinent Baseline in chronically-treated CTL animals (0.88 ± 0.12 cells/track) appears to be higher than at Baseline in drug-naïve CTL animals (0.66 ± 0.07 cells/track) and more similar to drug-naïve MAM animals (1.01 ± 0.10 cells/track) at Baseline. This could suggest that chronic NIC treatment elevates DA population activity observed in acute abstinence in CTL animals. Our baseline findings of DA population activity in both MAM and CTL animals are somewhat lower than prior published observations, though we have replicated the relative difference between these groups observed in other studies (Lodge and Grace 2007). Our results support further study of acute and chronic effects of NIC on neurophysiological disruptions in MAM animals in order to make more direct comparisons.

There are several key limitations to our study design that may be considered in the interpretation of our findings. We elected to use experimenter-administered rather than self-administered NIC in our study in order to precisely control the timing and quantity of NIC received by all animals. While the dose selected (0.3 mg/kg, s.c.) for chronic NIC treatment is a moderate NIC dose relevant to human consumption, intravenous NIC self-administration in Chronic NIC experiments would be more representative of the pharmacokinetics of smoking. Additionally, the nature of the electrophysiological recordings performed in these experiments precluded us from directly examining how changes in neuronal activity after NIC administration may impact behavioral deficits in the MAM model. Future experiments utilizing recording techniques in awake, behaving animals would critically inform the relationship between normalization of neuronal dysfunction and resulting behavioral outcomes. An additional point of consideration of these data is that our experiments pooled male and female subjects in each group but were not statistically powered to detect sex differences as a variable of interest. We did not anticipate sex differences in MAM rats, as prior studies of this question have demonstrated consistent behavioral impairments and neurophysiological disruptions between male and female rats, including alterations in hippocampal spike timing and PV interneuron expression (Perez et al. 2019; Hernan et al. 2018). Limited study of GD17 MAM administration in mice has indicated some sex differences in the resulting phenotype, though it is unclear if these are distinct to the species and if they may interact with effects of NIC (Chalkiadaki et al. 2019).

To date, no published study has examined the effects of NIC on neurophysiological dysfunction observed in the MAM model. However, a 2018 study by Neves and Grace demonstrating that α7 nAChR agonist administration can normalize elevated VTA DA population activity in MAM animals via action in vHipp led us to hypothesize that administration of NIC may

produce similar results (Neves and Grace 2018). NIC exerts effects on multiple neurotransmitter systems by binding with nAChRs expressed on both excitatory and inhibitory cells, producing modulatory effects throughout the brain that vary across states of nAChR activation and desensitization (Picciotto and Mineur 2014; Picciotto et al. 2008). Co-labeling experiments have indicated that both α4β2 and α7 nAChRs are highly expressed on parvalbumin-positive (PV) inhibitory interneurons in the hippocampus, and decreased expression of PV is a well-established finding in studies of post-mortem tissue in SCZ (Freedman et al. 2000). In addition, reductions in functional α7 nAChRs are also observed in multiple regions of post-mortem SCZ brains, including thalamus, frontal cortex, and hippocampus (Leonard et al. 2002; Freedman et al. 1995). Unpublished [$_{125}$I]-labeled α-bungarotoxin autoradiography data from our lab suggest that expression of functional α7 nAChRs is also reduced in the vHipp of male MAM animals (Appendix A). Together, these findings may suggest a mechanism by which activation of both high-affinity α4β2 and low-affinity α7 nAChRs by high NIC concentrations may serve to potentiate activation of these regions of reduced expression and thus mitigate imbalances in excitation and inhibition that produce behavioral and neurophysiological deficits.

Here, particularly, we proposed that activation of nAChRs on inhibitory interneurons of vHipp may serve to normalize increased activity of vHipp pyramidal cells and, in turn, restore proper inhibition of the NAcc-ventral pallidum (VP) GABAergic input, which normalizes the increased DA population activity observed in VTA. We thus hypothesized that we would observe NIC-induced decreases in firing rate of putative pyramidal cells and of VTA DA population activity. While we did find that both acute and chronic systemic NIC administration normalized hyperactivity in both vHipp and VTA in MAM animals as hypothesized, our results cannot confirm that these effects occur via direct action of NIC on nAChRs in vHipp and its resulting drive to

VTA inputs. The use of systemic NIC in these experiments was justified by its relevance to NIC intake in human smokers but does limit the potential to draw mechanistic conclusions from our findings. Additionally, higher-affinity non-α7 nAChRs are found throughout hippocampus, maintaining the possibility that the effects of NIC observed in our experiments are not due to action on α7 nAChRs in vHipp (Yakel 2012). However, results of prior clinical and preclinical studies illustrating the particular role of vHipp α7 nAChRs in SCZ-related neuronal dysfunction converge with our finding that the effects of NIC on VTA and vHipp neuronal activity were specific to MAM animals and not observed in CTL rats (Neves and Grace 2018; Kohlhaas et al. 2015; Freedman et al. 2000). This suggests a mechanism that is particular to the pathophysiology of MAM. Further experiments employing regional, nAChR subtype-specific antagonism could better elucidate the precise mechanism by which systemic NIC reduces both vHipp and VTA DA hyperactivity in MAM animals. Importantly, the potential for normalization of VTA activity via vHipp has implications for effects on other projection regions, providing an additional potential mechanism by which NIC may modulate behavioral deficits in SCZ. Further studies are necessary to better clarify the potential mechanistic link between NIC effects on neuronal activity in vHipp and VTA and how these may change over the course of repeated administrations.

A wide body of research indicates a promising role for NIC and nicotinic agonist drugs in mitigating cognitive and sensory gating dysfunction in SCZ. Elevated rates of smoking among SCZ patients are often attributed to a "self-medication hypothesis", proposing that NIC delivered through cigarettes reduces some cognitive or negative symptoms of the disease (Kumari and Postma 2005). Developing an understanding of this relationship and the role it may play in tobacco use is critical to reducing persistent tobacco-related health disparities among individuals with SCZ. Additionally, the effects of NIC as well as acute abstinence from NIC are important to consider in

future NIC reduction policy, as there may be disparate effects of lower NIC product standards on quality of life and function in patients (Khantzian 2016). Based on findings that α7 nAChR agonists may normalize elevated DA population activity in the MAM model via action in vHipp, our experiments sought to explore the potential relationship between this neurophysiological mechanism and the established behavioral effects of NIC. Our experiments confirmed that, like α7-specific agonists, systemic NIC administration can normalize elevated VTA DA population activity observed in MAM animals, and that this effect persists in chronically-treated animals. Additionally, we demonstrated that both acute and chronic NIC administration can also normalize hyperactivity in vHipp, suggesting a potential means by which a reduction of vHipp output may in turn reduce VTA DA population activity. Future work can build upon our findings through more direct mechanistic study of vHipp activity and resulting effects on VTA DA output and through experimental connection of these neurophysiological findings with relevant behavioral outputs. Our findings nonetheless are a valuable contribution to the growing understanding of the neurophysiological and behavioral effects of NIC in SCZ developed through studies in animal models of the disease.

5.0 GENERAL DISCUSSION

5.1 SUMMARY OF FINDINGS

The studies summarized in this document aimed to assess two prevailing hypotheses behind high rates of smoking observed in people with SCZ using the MAM rodent model, a well-validated animal model of key neurophysiological and behavioral elements of the disease.

In **Chapter 2**, we described a series of experiments comparing the primary reinforcing and reinforcement-enhancing effects of NIC in MAM and CTL animals using NIC self-administration. Ultimately, we sought to evaluate the hypothesis that SCZ pathophysiology, particularly as it relates to DA hyperactivity, confers increased NIC reward and leads to higher rates of smoking in SCZ. Our findings demonstrated that self-administration of NIC, across a range of doses or paired with other reinforcers, is not increased in the MAM model of SCZ (Weeks et al. 2020). These results failed to support a "reinforcement hypothesis" behind increased rates of smoking in SCZ.

The experiments in **Chapters 3 & 4** then assessed the effects of both acute and chronic NIC on several behavioral measures of cognitive dysfunction and on neurophysiological disruptions observed in the MAM model. We presented findings that NIC, administered both acutely and chronically, can normalize auditory PPI and NOR in MAM animals. These results illustrated that NIC can improve certain SCZ-relevant cognitive impairments and sensory gating deficits modeled by gestational MAM administration. Additionally, the findings presented in

Chapter 4 suggested that NIC administration normalizes neurophysiological disruptions as well. Both acute and chronic NIC reduced neuronal hyperactivity in VTA and in vHipp to levels comparable to CTL animals. Taken together, these findings lent support to the other prevailing theory behind increased smoking, a "self-medication" hypothesis. The finding that both acute and chronic NIC administration normalized SCZ-relevant behavioral and neurophysiological dysfunction in the MAM rodent model of SCZ suggests the potential for a mechanism by which NIC intake could improve symptoms in smokers.

5.2 RELEVANCE TO THEORIES EXPLAINING INCREASED SMOKING IN SCHIZOPHRENIA

5.2.1 Evaluating the "reinforcement hypothesis".

The "reinforcement hypothesis" of increased smoking in SCZ posits that high rates of heavy smoking among individuals can be attributed to altered reinforcement or reward dysfunction in the disease. This wider theory, however, can be approached from several angles. One interpretation is that NIC reinforcement is greater in SCZ smokers than in controls, likely as a result of higher NIC-stimulated mesolimbic DA release and an increased vulnerability to addiction (Chambers et al. 2001). Indeed, some behavioral evidence does support this idea of increased NIC reinforcement. Smokers with SCZ demonstrate a distinctly heavy pattern of smoking, consuming more cigarettes per day, taking more puffs per cigarette, and reporting higher levels of craving than nonpsychiatric control smokers (Tidey et al. 2005). Additionally, in behavioral economic analyses, SCZ smokers exhibited a higher intensity of cigarette demand than control smokers

(MacKillop and Tidey 2011). While SCZ smokers' demand for cigarettes was sensitive to hypothetical changes in cigarette price, this group reported significantly higher demand for cigarettes at hypothetical low prices and higher rates of consumption during an *ad libitum* smoking task than control smokers, suggesting some differences in smoking reinforcement. Smokers assessed on decisional balance indices to evaluate the degree to which they perceive benefits versus disadvantages of smoking also vary by psychiatric status. Interestingly, control smokers reported a near-balanced perception of pros and cons of smoking, while both depressed and SCZ smokers reported significantly greater benefits of smoking, even after controlling for nicotine dependence severity (Spring et al. 2003). This could support the theory that smoking is more reinforcing in SCZ smokers than in controls, but the finding that depressed and SCZ smokers reported similar perceptions of smoking-related benefits would suggest that this difference in reinforcement is not unique to SCZ pathophysiology. The experiments described in **Chapter 2** sought to directly compare NIC reward in CTL and MAM animals using intravenous self-administration, the gold standard in studies of NIC reinforcement (Ator and Griffiths 2003). Though elevated DA population activity is one of the primary physiological hallmarks of the MAM model, and that altered DA activity is thought to underlie increased sensitivity to NIC reward, no prior experiments had assessed NIC self-administration in this model. We found that self-administration of NIC, alone or in combination with a reinforcing VS, was not increased in MAM animals (**Chapter 2)**. This suggests that SCZ-related DA pathophysiology modeled by gestational MAM administration does not produce increased NIC reinforcement relative to controls.

However, the reinforcement hypothesis could also imply that NIC is more rewarding to SCZ individuals than other rewards, which are more weakly reinforcing than in controls, leading to higher rates of smoking. SCZ smokers assessed on a hypothetical choice task were more likely

than control smokers to select cigarettes over other rewards, such as eating a favorite candy or seeing a movie (Spring et al. 2003). Importantly, this difference was also observed in depressed patients, suggesting a behavioral mechanism not necessarily exclusive to SCZ symptomology. The results of experiments from **Chapter 2** lend some support to the hypothesis that rewarding effects of NIC are elevated relative to other reinforcers in SCZ. MAM animals self-administered fewer infusions paired with VS presentations than CTL animals at all doses of NIC besides 100 µg/kg, a relatively high dose. MAM animals' lower responding for the reinforcing VS than CTL animals in absence of NIC (0 µg/kg) and reduced response breakpoints for sucrose rewards could suggest reduced responsivity to non-NIC rewards in this group. The finding that self-administration of NIC alone was comparable between MAM and CTL animals across a range of doses in this study would in turn suggest that NIC reinforcement in MAM animals, while not greater than in CTL, is greater than reinforcement from non-NIC rewards.

A further possible interpretation of the reinforcement hypothesis is that this difference in valuation of NIC versus non-NIC rewards is related to symptoms of anhedonia in SCZ. Indeed, increased levels of negative symptoms in SCZ, such as flattened affect and anhedonia, have been associated with higher rates of smoking and less success in smoking cessation (Ameringer and Leventhal 2010; Dutra et al. 2012). Baseline NIC withdrawal severity and non-deprived urge to smoke were also positively correlated with blunted affect and anhedonia among SCZ smokers, but not controls (AhnAllen et al. 2012). Importantly, work studying the nature of anhedonia symptoms in SCZ has suggested that deficits are specific to anticipatory pleasure, and that experiences of consummatory pleasure seem to be comparable in SCZ patients and controls (Gard et al. 2007). Higher reports of craving and urge to smoke in SCZ smokers measured both in non-deprived and acutely abstinent conditions (Tidey et al. 2014) are thus interesting, as they reflect a more intense

anticipation of smoking without necessarily relating to the experience of smoking itself. This further suggests that, in SCZ smokers, NIC may be uniquely reinforcing relative to other rewards and that this may be related to symptoms of anhedonia. A study of reward-based learning in SCZ and control smokers found that, while both groups were comparable in patterns of reward-based learning, decreased responsiveness to rewards was associated with higher dependence in SCZ smokers only (AhnAllen et al. 2012). This finding could imply that SCZ smokers with reduced hedonic function or capacity are more reliant on NIC for normalization of reward system functioning, and thus more dependent on NIC. The reinforcement-enhancing effects of NIC could contribute to this relationship by modulating responses to non-NIC rewards (Rupprecht et al. 2015), essentially normalizing blunted reward responses in SCZ. However, in briefly NIC-deprived, nonpsychiatric control smokers, higher reports of cigarette craving have been associated with lower responsivity to monetary rewards (Peechatka et al. 2015). In SCZ patients experiencing symptoms of anhedonia, then, NIC dependence and craving could perpetuate a relationship between smoking, NIC reinforcement, and reward responsivity by further reducing responses to non-NIC rewards. Taken together, the findings described above suggest that a "reinforcement hypothesis" may not necessarily imply that individuals with SCZ are more likely to smoke because they experience greater NIC reward than controls, and that NIC reinforcement in SCZ may be associated with symptom severity.

5.2.2 Cognitive and neurophysiological effects of nicotine as evidence for "self-medication".

The second popular theory underlying increased smoking among SCZ patients is a "self-medication hypothesis". This suggests that individuals with SCZ smoke as a means of managing or relieving cognitive and/or negative symptoms of the disease with NIC (Kumari and Postma

2005). As reviewed in **Chapter 3,** numerous studies of subjects with SCZ have demonstrated improvements in sensory gating deficits and in impaired cognitive domains such as attention and working memory after administration of NIC. For example, SCZ smokers administered NIC nasal spray or allowed to smoke prior to testing demonstrate increased levels of auditory PPI relative to both SCZ nonsmokers and briefly deprived smokers (Hong et al. 2008; Kumari et al. 2001). Additionally, when allowed to smoke *ad libitum,* SCZ smokers exhibit increased PPI to levels comparable to control smokers (Woznica et al. 2009). NIC-induced improvements in cognitive domains have also been reported, suggesting further motivation for self-medication. For example, administration of a transdermal NIC patch significantly improved attentional performance on the Continuous Performance Test in both SCZ and control nonsmokers, and improved impulsive responding to a greater degree in SCZ subjects (R. S. Barr et al. 2008). The findings of our experiments detailed in **Chapters 3 & 4** support elements of the self-medication hypothesis by providing both behavioral and neurophysiological evidence of NIC-induced improvements in a model capturing certain key system dysfunctions in SCZ. We found that acute administration of NIC mitigated PPI deficits and impairments in the NOR task in MAM animals and normalized increased activity in VTA and vHipp in these animals. Importantly, we also found that MAM and CTL animals did not differ in behavioral performance after chronic treatment with NIC. This was a critical measure, as some lines of evidence suggest that NIC-induced improvements in smoking patients are merely a result of relief from withdrawal, or that NIC does not improve symptom measures in smokers. For example, an assessment in multiple indices of cognitive function before and after the use of NIC gum demonstrated improved measures of attentional function only in nonsmokers, with no benefit in smokers (Harris et al. 2004). Another study of smokers found that overnight abstinence exacerbated deficits in spatial working memory and attention in SCZ

smokers, and that smoking resumption increased performance in these areas (Sacco et al. 2005). Our experiments found that chronically NIC-treated animals, when measured at a 24-hr abstinence period, did not differ in behavioral or neurophysiological measures. While the study design precludes us from directly comparing these measurements to those obtained in the acute administration experiments, our findings suggest that this does not reflect a decline in CTL animals' performance and function, but rather a persistent normalization of these indices in MAM animals.

Our studies, however, cannot provide insight into if or how the normalizing effects of NIC on behavioral and neurophysiological dysfunction in SCZ may motivate smoking as a means of "self-medication". Few studies assess self-reported motivators for smoking among SCZ patients, but the most commonly cited reasons for smoking in these reports are calming or sedative effects of smoking, improvement of negative symptoms, and relief of craving (Gurpegui et al. 2007; Forchuk et al. 2002). It is possible, then, that these reported motivators for smoking, in combination with modest improvements in sensory gating impairments and cognitive dysfunction, produce a general state of "feeling better" that may drive smoking among SCZ individuals. That is, perhaps some of the effects of NIC on SCZ symptomology observed in experimental measures are not clearly discernible in daily life, and that self-medication through smoking reflects a general means of "feeling better" without necessarily targeting specific cognitive deficits.

5.2.3 A potential merging of the reinforcement and self-medication hypotheses.

Despite their considerable differences, the two most prominent hypotheses explaining increased smoking in SCZ are not mutually exclusive, and could, in fact, come together into a broader hypothesis encompassing elements of both theories. Based on the evidence described

above, it is possible that heavy smoking in SCZ is driven by uniquely reinforcing effects, in that "feeling better" after smoking provides a form of negative reinforcement. This general improvement of some symptomology could perpetuate smoking behavior as a means of relief from unpleasant states related to SCZ, even if patients do not consciously consider this action as a means of "self-medication". Additionally, the potential effects of NIC intake on negative symptom severity in SCZ smokers could impact cognitive symptoms as well. Negative symptoms affecting reward, such as anhedonia and avolition, have an overlapping impact on overall cognitive function, as motivation, effort allocation, and value representation are processes necessary to many cognitive demands (Robison et al. 2020). A study of SCZ smokers and nonsmokers demonstrated a correlation of performance on the Iowa Gambling Task (IGT), a measure of reward-based learning, and the Wisconsin Card-Sorting Task, a test of cognitive flexibility. This finding could reflect associated levels of function in reward processing and executive functioning (Yip et al. 2009). Negative reinforcement through relief of withdrawal symptoms is also considered a significant motivator of continued smoking behavior in the general population (Robinson et al. 2012). While this could be an important additional factor in driving persistent smoking in SCZ smokers as well, as patients report more severe withdrawal symptoms after 72-hr abstinence than control smokers (Tidey et al. 2014), it does not preclude the possibility of reinforcement related to cognitive and affective states as a motivator of NIC intake.

Our studies indicate that acute NIC administration can improve cognitive impairments and sensory gating deficits and normalize abnormal neuronal activity in the MAM rodent model of SCZ. Our findings also suggest that these effects are lasting and persistent with repeated treatments, as animals treated chronically with NIC demonstrate continued normalization of these measures. Importantly, these results do not illustrate further impairment of cognitive function after

24-hr abstinence in chronically treated animals. When measured at this briefly abstinent baseline, MAM and CTL animals were comparable in behavioral and neurophysiological measures, suggesting that overnight NIC abstinence may not produce an exacerbation of SCZ-related dysfunction and that NIC may have sustained positive effects on behavioral and neurophysiological disruptions in this model. Overall, the results of our experiments provide support for some aspects of both the reinforcement and self-medication hypotheses. While NIC is not more reinforcing in MAM animals than controls, our findings suggest that NIC may be uniquely reinforcing relative to other rewards in this model. Furthermore, the findings described in **Chapters 3 & 4** indicate that both acute and chronic NIC administration can normalize SCZ-related behavioral and neurophysiological dysfunction modeled by gestational MAM administration. Taken together, these results could provide evidence for a merged hypothesis, where the effects of NIC on reward-related deficits as well as cognitive functioning and underlying neurophysiological processes may reinforce continued smoking behavior in SCZ.

5.3 CONTRIBUTION TO PROPOSED MECHANISM OF NICOTINE'S EFFECTS IN SCHIZOPHRENIA

Nicotinic receptors have long been of interest in the study of SCZ pathophysiology and development of potential therapeutics. Multiple lines of evidence suggest that the α7 nAChR subtype is of particular relevance to the disease. Briefly, α7 nAChRs are homomeric receptors with rapid desensitization kinetics and high permeability to Na^{+}, K^{+}, and Ca^{2+}. This high Ca^{2+} permeability is critical to the receptor's effects in depolarizing postsynaptic cells, regulating neurotransmitter release, and initiating downstream cellular processes. These receptors have a

lower affinity for NIC than most other nAChRs and thus require high concentrations of the drug, such as those achieved through patterns of intense, heavy smoking observed in SCZ smokers, to be activated (Corradi and Bouzat 2016). α7 nAChRs are particularly abundant in regions implicated in memory and cognitive function, including hippocampus and cortex, and play a critical role in modulating synaptic transmission throughout the brain (Lendvai et al. 2013). Importantly, post-mortem studies of patient tissue have revealed a significant reduction in binding of [^{125}I]-labeled α-bungarotoxin in the hippocampus, frontal cortex, and thalamus of SCZ brains, indicating a reduction in α7 subunit expression in these regions (Guan et al. 1999; Freedman et al. 1995; Court et al. 1999). Our preliminary evidence from [^{125}I]α-bungarotoxin autoradiography suggests that a decrease in α7 nAChR expression is also observed in the vHipp of male MAM animals (Appendix A). Genetic evidence associates these abnormalities in α7 nAChR function with wider implications for SCZ prevalence, symptom severity, and smoking behavior. Abnormalities in CHRNA7, the gene encoding the α7 nAChR subunit, are reliably linked to the P50 sensory gating deficit in patients and first-degree relatives, and further studies have demonstrated the gene's association with PPI, visual information processing, and episodic memory dysfunction (Bakanidze et al. 2013; Freedman 2014). CHRNA7 variants associated with smoking prevalence, both overall and in SCZ patients in particular, have also been identified (Stephens et al. 2012; De Luca et al. 2004).

Evidence also suggests that expression and function of α4β2 nAChRs may be altered in SCZ, as autoradiographic analyses have demonstrated decreased levels of these receptors in hippocampus, striatum, and cortex in patient tissue relative to controls (Ripoll et al. 2004). In addition to their role in modulating neurotransmitter release throughout the brain, similarly to α7 nAChRs, these receptors are also particularly implicated in the reinforcing effects of NIC. α4β2

nAChRs are prominently expressed on presynaptic terminals of DA neurons, and nearly 100% of neurons in VTA express mRNA for the α4 and β2 subunit (Klink et al. 2001). Systemic or intra-VTA administration of DHβE, an antagonist selective for α4- and β2-containing nAChRs, has been demonstrated to decrease NIC self-administration and minimize NIC-induced lowering of intracranial self-stimulation thresholds in rats (Fowler et al. 2008), suggesting that stimulation of α4β2 nAChRs is critical to the effects of NIC on brain reward systems. Additionally, while significant upregulation of high-affinity nAChRs is consistently observed in control smokers due to chronic NIC exposure, this process may be altered in smokers with SCZ. A study of postmortem tissue from SCZ subjects and nonpsychiatric controls demonstrated that binding of the high affinity receptor ligands [^{3}H]-epibatidine and [^{3}H]-nicotine in cortex, hippocampus, and caudate increased as a function of smoking status in both groups, though to a significantly lesser degree in samples from SCZ patients (Breese et al. 2000). These findings suggest that decreased levels of α4β2 nAChR expression, both in NIC-naïve and smoking SCZ subjects, could contribute to differences in responses to stimulation by NIC as well as endogenous ACh. Despite the wealth of research into the potential mechanistic role of nAChR stimulation in SCZ and its relationship to smoking, this relationship remains largely unexplored in the MAM model. Recent work by Neves & Grace (2018) demonstrated that administration of an α7 nAChR agonist, either systemically or directly into the vHipp, normalized the elevated VTA DA population activity observed in MAM animals and had no effect on CTL animals. This finding suggests a critical role for α7 nAChR activation in modulating abnormal vHipp drive to the GABAergic NAc-VP input to VTA, and that this effect may be unique to the altered hippocampal-striatal activity observed in MAM animals (Lodge and Grace 2007). The experiments described in **Chapter 4** confirmed a role for nicotinic stimulation in modulating vHipp output as well as VTA DA population activity. We

demonstrated that systemic NIC administration can normalize the elevated firing rate observed in putative pyramidal neurons of the vHipp as well as elevated VTA DA population activity in MAM animals. However, because NIC broadly activates nAChRs, these results do not provide insight into specific nAChR subtypes responsible for these actions. These and other findings suggest roles of multiple nAChR subtypes, including high-affinity subtypes such as α4β2 nAChRs, in modulating SCZ pathophysiology modeled in MAM animals. Indeed, while α7 nAChRs are critical in these mechanisms, numerous receptor subtypes are at play in both neurophysiology and related behavioral output. For example, NIC administration improves learning in WT but not α7 null mice, but α7 agonists do not improve learning (Milienne-Petiot et al. 2018). This finding could illustrate that α7 nAChR stimulation is likely necessary, but not sufficient, for normalization of cognitive deficits.

As mentioned previously, reinforcement-based explanations for increased smoking in SCZ have been largely associated with alterations in the mesolimbic reward system. Endogenous cholinergic inputs from pedunculopontine tegmentum (PPTg) to VTA are critical in modulating DA neuronal activity, as nAChRs are expressed not only in VTA DA cells, but on glutamatergic and GABAergic cells of the VTA as well (Mansvelder et al. 2002). As previously discussed, high-affinity β2 subunit-containing receptors in this region are known to be crucial in the rewarding effects of NIC, though α7 nAChRs are also expressed in VTA. Cholinergic inputs from PPTg, as well as glutamatergic afferents also regulated by nAChR activity, are critical in transitioning DA neurons from tonic to burst firing patterns (Leslie et al. 2013). In the state of elevated DA population activity observed in the MAM model, then, one might expect a greater NIC-induced excitation in VTA DA neurons, as more spontaneously active neurons would be available to respond (Lodge and Grace 2009). However, the results of experiments in **Chapter 4** were

relatively surprising in this respect, as acute NIC administration did not increase bursting or overall firing rates of spontaneously active VTA DA neurons in MAM or CTL animals. While it is possible that a transient initial increase in activity after NIC administration was briefly observed and then normalized due to receptor desensitization, α6α4β2-containing nAChRs in VTA remain persistently activated for minutes after desensitization (Leslie et al. 2013) This finding could suggest that action of NIC on nAChRs in other regions may have prevented this increase.

Afferents from vHipp are additional critical sites of nicotinic modulation that can impact activity throughout the brain. Evidence from the MAM model suggests that increased activity of vHipp excitatory projections to the NAcc-VP circuit disinhibits VTA DA cells and produces the elevated DA population activity thought to contribute to positive-like symptoms in these animals (Lodge and Grace 2007). Selective loss of a specific population of inhibitory interneurons expressing the calcium-binding protein parvalbumin (PV) appears to be key in this vHipp hyperactivity, as lentiviral knockdown of PV expression in vHipp produces increases in vHipp firing rates comparable to those observed in MAM animals (Boley et al. 2014). Importantly, decreased PV expression is consistently observed in PFC and vHipp of postmortem SCZ tissue (Beasley and Reynolds 1997; Zhang and Reynolds 2002), a finding which is replicated in the MAM model and is likely responsible for impaired functional coupling between these regions during cognitively demanding tasks (Lodge et al. 2009). Because afferents of PFC and hippocampus project to diverse regions of the brain, the disruption in inhibitory and excitatory balance resulting from PV interneuron dysfunction has broad functional implications. We propose that the action of NIC administration in normalizing vHipp and VTA neuronal activity in MAM animals observed in our studies may occur via potentiation of inhibitory activity in the vHipp (Figure 12). Both α7 and α4β2 nAChRs are expressed on GABAergic interneurons of the vHipp,

including PV interneurons (Alkondon and Albuquerque 2002). These inhibitory cells potently modulate pyramidal cell spiking, and activation and desensitization kinetics of the nAChRs expressed on these neurons can thus have major effects in modulating inhibition and disinhibition of pyramidal cells (Ferguson and Gao 2018). Interestingly, high plasma NIC levels observed in SCZ smokers (McKee et al. 2009) may be particularly effective in activating low-affinity α7 nAChRs in addition to higher affinity receptors. Additional evidence suggests that, in addition to desensitization following activation, these receptors desensitize with sustained exposure to subthreshold agonist concentrations (Olale et al. 1997). This could suggest that constant low levels of NIC may fail to produce any activation of α7 nAChRs, while NIC levels associated with distinctly heavy smoking patterns may produce unique effects in activating these receptors.

Prior studies have demonstrated that α7 nAChR stimulation in vHipp is sufficient to normalize elevated DA population activity (Neves and Grace 2018), and our experiments suggest that activation of multiple nAChR subtypes by systemic NIC administration produces normalization of both elevated DA population activity and vHipp hyperactivity in MAM animals. Importantly, these effects were not observed in control animals, suggesting a mechanism unique to MAM pathophysiology. Reductions in α7 nAChR expression in this region demonstrated by our lab (Appendix 1), as well as highly documented PV interneuron dysfunction in MAM animals (Gill and Grace 2014), are two potential candidate mechanisms by which nicotinic activation may uniquely potentiate vHipp inhibitory balance in MAM animals. Our autoradiography data cannot discern whether this reduction in α7 nAChR expression is specific to PV interneurons. However, direct comparisons of α7 receptor concentrations in control animals showed minimal expression in CA1 pyramidal cells, with high expression in interneurons (Frazier et al. 1998), making this a likely possibility. Additionally, while our electrophysiological experiments focused on activity in

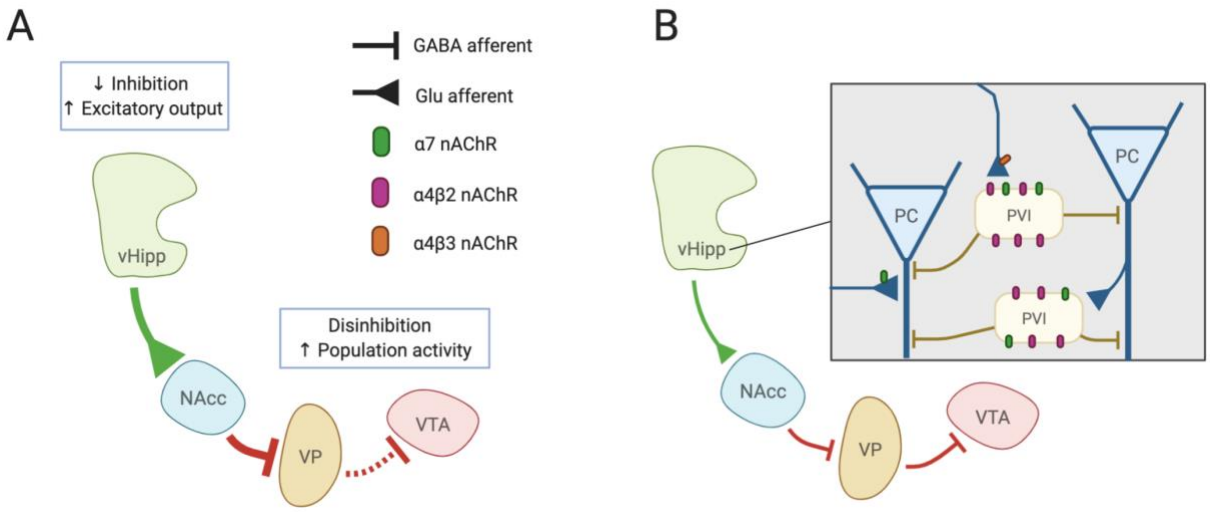

Figure 12: Schematic of proposed mechanism of NIC in vHipp

(A) vHipp-NAcc-VP circuit disruption leading to elevated VTA DA population activity. Inhibitory dysfunction in vHipp (decreased PV expression/reduced activation of inhibitory interneurons due to reduced expression of α7 and α4β2 nAChRs) contributes to elevated excitatory output to NAcc. Inhibitory projections from NAcc which typically modulate VP inhibition and disinhibition of VTA are thus also increased. This produces a constant state of disinhibition to VTA (*dashed line*) and, in turn, abnormally high tonic DA activity. (B) Proposed action of NIC in vHipp (*inset).* NIC activation of α7 and α4β2 nAChRs on inhibitory interneurons, including the reduced population of PV interneurons, potentiates GABA input to pyramidal cells, normalizing activity. α7 nAChRs expressed on presynaptic terminals of glutamatergic inputs to pyramidal cells desensitize quickly, reducing their potentiation of transmitter release. α4β2 and α4β3 nAChRs on inhibitory interneurons and on presynaptic terminals of glutamatergic inputs exciting them desensitize more slowly, favoring sustained potentiation of inhibitory input to pyramidal cells. *Pyramidal cells, PC; parvalbumin-positive interneuron, PVI.*

vHipp and VTA, it is important to note that the effects of systemic NIC administration are relevant throughout the brain. Modulation of vHipp excitation has important implications for excitation/inhibition balance between hippocampus and PFC, as well as other hippocampal projection regions. Nicotinic stimulation undoubtedly has direct neuronal effects in PFC as well, where similar decreases in PV interneuron function are observed in both humans and MAM animals (Beasley and Reynolds 1997; Gastambide et al. 2012), though detailed conjectures regarding effects in this region are beyond the scope of our experimental results. However, modulation of excitation/inhibition balance within and between these regions could provide a general mechanism by which NIC administration improves sensory gating and cognitive deficits in the MAM model, as described in **Chapter 3.** Prior evidence has suggested that α4β2 nAChR agonism is sufficient to potentiate GABAergic activity and reverse impairments in a multisensory integration task in rodents with experimentally inhibited PV interneurons in orbitofrontal cortex (Cloke et al. 2016), which suggests the potential of a similar mechanism in the behavioral effects of NIC we observed in MAM animals.

While some clinical evidence suggests that acute effects of NIC on cognitive function and sensory gating impairments in SCZ smokers may reflect a reversal of withdrawal-induced deficits rather than a positive effect, our findings did not demonstrate such an impact on baseline function after brief NIC withdrawal. After chronic NIC treatment, we demonstrated that MAM and CTL animals were comparable in both behavioral and electrophysiological measures at a 24-hr abstinence baseline. These findings do not rule out the possibility of such an effect, as our experiments utilized a daily injection protocol that may not completely capture the patterns of NIC exposure and withdrawal symptoms experienced by human smokers. However, they do suggest that repeated exposure to moderate NIC doses does not necessarily result in compensatory

neurophysiological alterations that negatively impact function in the acute absence of NIC. Interestingly, some evidence suggests that nAChR upregulation after chronic NIC exposure may be less robust in brains of SCZ smokers than of controls. An imaging study using radiolabeled β2 nAChR agonist drug found lower receptor availability in SCZ smokers than in control smokers in frontal cortex, parietal cortex, and thalamus (D'Souza et al. 2012). Additionally, studies of postmortem tissue found significantly lower upregulation of α4β2 binding in SCZ smokers than control smokers in hippocampus, cortex, and caudate (Breese et al. 2000). Our findings, when placed in the scope of existing literature, suggest effects of NIC in MAM pathophysiology that are distinct from those observed in controls, and likely illustrate the potential of NIC potentiation of inhibitory balance in MAM animals as a means of normalizing SCZ-related behavioral and neurophysiological perturbations. Further studies can better elucidate the detailed neurophysiological basis of these effects as well as their direct connections to related behaviors.

5.4 LIMITATIONS AND CONSIDERATIONS

Several key considerations must be made in evaluating the data presented here. First and foremost, experiments utilizing the MAM rodent model of SCZ, or any animal model of SCZ, will always be limited by the fact that SCZ is a distinctly human disease. There is no single known etiology of SCZ, precluding the precise replication of the disease state in an animal model, and it is characterized largely by its symptoms. Though we cannot experimentally study psychosis in animals, we are able to model neurophysiological correlates of psychosis and other disease-relevant system features. Importantly, the MAM rodent model has core features of face validity in modeling SCZ, including measurable positive-like symptoms reflecting abnormal DA function

such as sensory gating dysfunction, as well as cognitive impairments and limited negative symptom analogs (Modinos et al. 2015). Additionally, this model has a relatively high degree of predictive validity, as antipsychotic drugs effectively ameliorate analogs of psychosis (Sonnenschein and Grace 2020), and induction of the model through neurodevelopmental disruption informs some understanding of disease etiology. Our studies of NIC effects in the MAM model, however, are limited in modeling human NIC use in several key respects. Firstly, our self-administration studies described in **Chapter 2**, while effective in assessing reinforcing effects of NIC alone, cannot capture the full spectrum of experiential and psychosocial factors that may contribute to continued smoking in human SCZ patients. Additionally, if smoking in SCZ patients is reinforced by the effects of NIC intake on "feeling better" rather than the rewarding effects of NIC, it would not be possible to assess this type of motivation in an animal model, as we cannot necessarily discern if "feeling better" (i.e. ameliorating some negative/cognitive symptoms, even if captured in the model) would drive self-administration behavior. Furthermore, the experiments of **Chapters 3 & 4** utilized a drug administration protocol of daily, experimenter-administered subcutaneous injection of NIC at a single moderate dose in order to control for the specific timing and quantity of NIC each animal received. Indeed, NIC exposure in this injection protocol is not equivalent to the consistent, repeated dosing throughout the day experienced by heavy smokers, and 24-hour abstinence from these injections undoubtedly differs from an overnight withdrawal baseline typically measured in studies of human smokers. While the doses utilized in our experiments were sufficient to normalize behavioral and electrophysiological dysfunction in MAM animals, further exploration of dose-response relationships for NIC in these measures are warranted.

Another potential factor for consideration is that most SCZ smokers studied in clinical experiments are taking APDs at the time of study, which was not the case for animals used in our experiments. Our findings do provide useful insight about the effects of NIC in unmedicated smokers with SCZ as well as potential effects on cognitive and negative symptoms, which are not effectively managed with APDs (Goff et al. 2011). However, it is possible that APD treatment status could produce alterations that modulate NIC reinforcement, affecting self-administration, or have system-wide modulatory implications for effects on cognitive deficits and neurophysiological perturbations in the MAM model. Similarly, levels of monoamine oxidase-A (MAO-A) and MAO-B activity in the brains of smokers are inhibited by approximately 30-40% due to an unidentified, non-NIC constituent of cigarettes (Lewis et al. 2007). Comparable pharmacological MAO inhibition has been shown to increase NIC self-administration in control rats (Smith et al. 2015) and MAM rats (Weeks, unpublished), though further potential effects on cognitive and neurophysiological function have not been assessed. Future experiments could explore what, if any, effect APD treatment and MAO inhibition may have on the behavioral and neurophysiological effects of NIC observed in our studies.

An additional important technical limitation to consider in evaluating our experimental findings is the dissociation of our behavioral and electrophysiological experiments. In separate experiments, we determined that acute and chronic NIC can normalize both behavioral and neurophysiological perturbations observed in the MAM model. Because of this separation, our utilization of extracellular recording in anesthetized animals precludes any conclusions directly connecting the normalization of elevated vHipp and VTA activity with normalization of cognitive and sensory gating impairments. Ultimately, because systemic NIC administration was utilized, we also cannot draw any certain conclusions regarding the precise mechanism by which NIC

administration normalized vHipp activity due to distribution of nAChRs throughout the brain. Thus, while the observed effects on activity in vHipp likely have implications for the balance of activity throughout the brain, we cannot determine precisely how normalization of vHipp activity and resulting impact on afferents from this region may translate to behavioral outputs. Direct stimulation of nAChRs in other regions, such as PFC, are also likely to play a role in the effects of NIC on NOR and PPI. Regionally specific pharmacological manipulation and electrophysiological recording in awake, behaving animals would be a critical step in ascertaining possible connections between our behavioral and neurophysiological observations.

5.5 IMPLICATIONS FOR PHARMACOTHERAPIES AND SMOKING CESSATION IN SCHIZOPHRENIA

Our studies sought to explore the neurophysiological and behavioral effects of smoking-relevant doses of NIC in a well-validated animal model of SCZ in order to assess how these effects may influence heavy smoking in SCZ patients. An understanding of the impact of NIC in daily functioning of SCZ smokers is critical to the development of successful cessation strategies as well as potential pharmacotherapies for negative and cognitive symptoms of the disease. Our findings suggested that acute systemic administration of NIC can normalize impairments in PPI and NOR, as well as neuronal hyperactivity in vHipp and VTA, observed in MAM animals. Importantly, we also found that this normalization appears to persist in animals chronically treated with NIC, even when measured at a 24-hr abstinence period, a finding which may have important implications for smoking cessation strategies as well as potential NIC reduction policy. Smokers with SCZ are consistently less successful in smoking cessation attempts despite considerable motivation to quit

(Cather et al. 2017), and this difference is often attributed to particularly severe withdrawal symptoms in this population. In a study of brief abstinence in smokers with SCZ and nonpsychiatric controls, SCZ subjects reported more severe cigarette craving and withdrawal symptoms throughout the 72-hr abstinence period. Moreover, this group reported greater NIC preference after abstinence and lapsed back to smoking significantly sooner than controls (Tidey et al. 2014). Some evidence also suggests that withdrawal may exacerbate cognitive impairments in SCZ smokers. In a study of VSWM task performance, 1-week abstinence improved performance in control smokers, but further impaired smokers with SCZ (George et al. 2002). However, other studies have demonstrated a lack of effect of both brief and prolonged abstinence on cognitive performance in SCZ smokers (Boggs et al. 2018). Our findings of similar performance in briefly abstinence CTL and MAM animals suggest that exacerbation of SCZ-related dysfunction, at least in the short term, may not be a primary component of particularly severe withdrawal symptoms in this population. This parallels findings that SCZ smokers are comparable to controls in effects of smoking abstinence and resumption on attentional performance, but report significantly higher withdrawal symptoms than controls (Hahn et al. 2013). Implications for negative symptom severity also could not be assessed in our experiments, which remains a possible component of persistent smoking in SCZ.

Understanding the effects of chronic NIC use and abstinence on functioning in SCZ smokers is particularly critical to the development of a reduced-NIC product standard aimed at reducing smoking, as disparate effects of NIC reduction could drive compensatory smoking in this population. However, trials of very-low-NIC-content (VLNC) cigarettes in smokers with SCZ have yielded promising results. A six-week trial of VLNC cigarettes in control and SCZ smokers demonstrated that, relative to usual brand smokers, those on VLNC performed more poorly on

measures of attention, working memory, inhibitory control, and processing speed regardless of psychiatric status. However, this difference was not observed in SCZ smokers given NIC replacement therapy (NRT) while smoking VLNC cigarettes, suggesting that NRT may effectively preserve cognitive functioning (AhnAllen et al. 2015). Another trial of VLNC versus normal NIC content cigarettes in SCZ smokers demonstrated that those in the VLNC condition smoked fewer cigarettes per day, had lower breath carbon monoxide levels, and comparable psychiatric symptoms to those in the normal NIC condition. Importantly, however, total NIC exposure did not differ between conditions, which suggests that smokers in the VLNC condition likely supplemented with alternative sources of NIC (Tidey et al. 2019). These findings suggest that SCZ smokers would likely respond to reduced NIC product standards in a similar manner to nonpsychiatric controls, but that alternative sources of NIC, such as NRT or e-cigarettes, may be an important tool in maintaining abstinence. Additionally, while it cannot be addressed in animal studies, it is key to acknowledge the unique psychosocial barriers to cessation faced by this population, such as a lack of provider support. In addition to the use of NRT or alternative NIC sources in smoking cessation, evidence suggests that integration of provider and peer support is particularly necessary in achieving cessation among SCZ smokers (Cocks et al. 2019).

The effects of NIC on measures of sensory gating and cognitive impairment observed in our studies also offer important insight to the development of potential pharmacotherapies for cognitive symptoms in SCZ. Despite the wealth of research largely focused on the α7 nAChR as a therapeutic target, there has been little success in drug development. The receptor's unique traits make it a challenging target for agonist drugs, as activation of the receptor produces rapid and profound desensitization, limiting the efficacy of sustained drug exposure. Initial clinical studies of α7 agonists and partial agonists have shown some significant improvements in cognitive

performance and sensory gating measures after acute administration. Efforts to develop an extended-release α7 nAChR agonist have found little efficacy in increasing cognitive performance in smokers or nonsmokers (Kem et al. 2018). While no α7 nAChR drug has succeeded in larger Phase III trials, development of potential α7 ligands, particularly positive allosteric modulators (PAMs) of the receptor, continues as a challenging avenue in improving cognitive symptoms of SCZ (Tregellas and Wylie 2019; Freedman 2014). Our findings highlight the potential utility of cognitive symptom pharmacotherapies targeting multiple nAChR subtypes rather than a focus on the α7 nAChR alone. The effectiveness of both acute and chronic NIC in normalizing behavioral and neurophysiological disruptions in MAM animals suggest a benefit of general nicotinic stimulation in the SCZ pathophysiology modeled by gestational MAM. This approach may have relevance to the development of more effective pharmacotherapies both for cognitive dysfunction and smoking cessation in SCZ patients. As described previously, NRT is generally promising as a smoking cessation aid in SCZ smokers and does not present adverse neuropsychiatric side effects (Shawen and Drayton 2018). Drugs aimed at aiding cessation such as varenicline, a full agonist of α7 nAChRs and partial agonist of α4β2 nAChRs, can have limited efficacy in patients with higher negative symptom severity (Dutra et al. 2012). However, double-blind studies do suggest that varenicline is more effective than placebo in significantly decreasing NIC intake and smoking urges among individuals with SCZ, though it does not differ from placebo in effects on psychiatric symptom severity (Smith et al. 2016). Stimulation of multiple nAChR subtypes at a level similar to NIC administration may thus be important for optimal efficacy in improving SCZ negative and cognitive symptomology and minimizing adverse effects of NIC withdrawal in this population.

5.6 FINAL CONCLUSIONS

In summation, the findings of our studies suggest that SCZ pathophysiology modeled by GD17 MAM administration does not produce increased NIC reinforcement relative to controls, suggesting that greater NIC reward alone is likely not a driver of increased smoking in SCZ. Our further studies demonstrated that acute and chronic systemic administration of NIC can normalize SCZ-relevant sensory gating and cognitive impairments observed in MAM animals. Additionally, acute and chronic NIC administration normalized elevations in VTA DA population activity, as well as the elevated vHipp neuronal activity thought to drive abnormal DA activity. Together, these findings suggest that nicotinic stimulation throughout the brain can have positive effects on behavioral dysfunction in the MAM model, as well as the imbalance in excitatory and inhibitory neuronal activity thought to underlie such dysfunction. These results provide evidence that effects of NIC on cognitive and negative symptoms of SCZ could be a potential driver of continued heavy smoking in SCZ. Importantly, these studies suggest the utility of future studies better clarifying the connection between NIC use and SCZ symptomology and elucidate pathways for future exploration of this relationship. We hope that such understanding can inform development of more efficacious pharmacotherapies for cognitive dysfunction in SCZ, as well as more targeted strategies for smoking cessation in this population.

APPENDIX A AUTORADIOGRAPHIC ANALYSIS OF α7 NACHR EXPRESSION

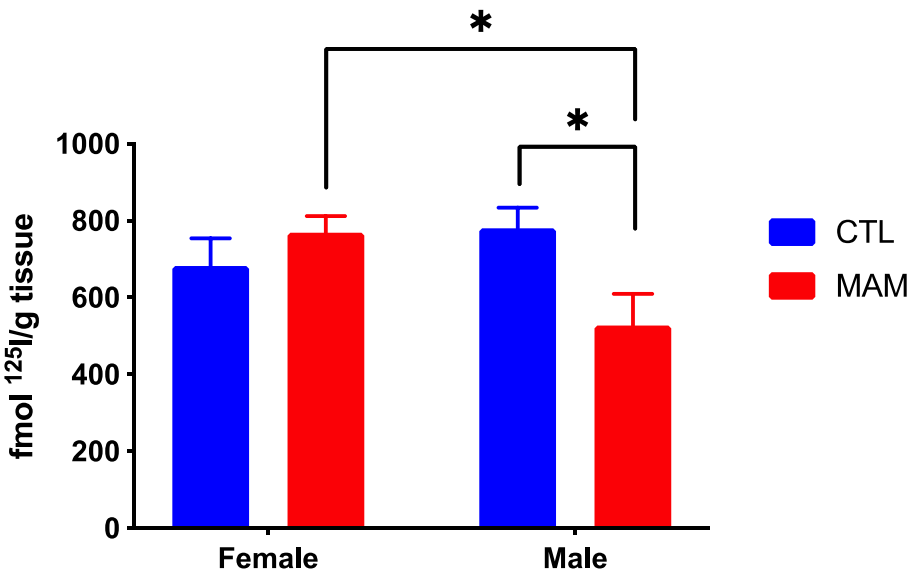

Figure 13: Reduced expression of vHipp alpha7 nAChRs in male MAM rats

Quantitative receptor autoradiography conducted using [$_{125}$I]-α-bungarotoxin in slices from brains of MAM and CTL rats demonstrated a sex-specific reduction in α7 receptor expression in vHipp of male MAM animals. Two-way ANOVA of [$_{125}$I]-α-bungarotoxin binding demonstrated a significant Group x Sex interaction ($p < 0.05$). Post-hoc t-tests demonstrated that binding of [I$_{125}$]α-bungarotoxin in vHipp was significantly lower in male MAM animals than both male CTL animals and female MAM animals. *$p < 0.05$.

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