Neurodevelopmental animal models of schizophrenia-like behaviours: Behavioural and neurochemical consequences of maternal immune challenge and prenatal NMDA receptor blockade

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A Thesis in the Department of

Psychology

Presented in Partial Fulfillment of the Requirements

for the Degree of Master of Arts (Psychology) at

Concordia University

Montreal, Quebec, Canada

August 2014

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

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Entitled: Neurodevelopmental animal models of schizophrenia-like behaviours: Behavioural and neurochemical consequences of maternal immune challenge and prenatal NMDA receptor blockade

and submitted in partial fulfillment of the requirements for the degree of

Master of Arts (Psychology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Date August 27, 2014

Abstract

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Concordia University, 2014

Schizophrenia is a severe psychiatric illness characterized by positive, negative, and cognitive symptoms. The observation that the onset of clinical symptoms is often in early adulthood has led to the neurodevelopmental hypothesis which posits that schizophrenia results from altered developmental processes beginning long before onset of clinical symptoms. The present thesis aimed to evaluate two neurodevelopmental animal models of schizophrenia-like behaviours. Chapter one explored the epidemiological association between maternal infection and schizophrenia by using a well-validated animal model of maternal viral infection. Pregnant Wistar rats were injected with the viral mimic polyriboinosinic-polyribocytidilic acid (poly I:C) and juvenile and adult offspring were assessed for altered novel object preference (NOP), sensitivity to the locomotor-activating effects of amphetamine, and deficits in preattentive sensorimotor gating mechanisms. Our findings suggest that prenatal poly I:C did not result in the expected behavioural deficits in adulthood. Our findings challenge the robustness of the poly I:C model. In chapter two, we examined behavioural and

neurochemical consequences of prenatal blockade of the glutamate receptor, N-methyl-D-aspartate (NMDA), in juvenile and adult offspring. Pregnant Long-Evans rats received daily injections of saline or the NMDA receptor antagonist, MK-801. Offspring were exposed to a battery of behavioural tasks. The most dramatic of our findings were the altered NOP, hyposensitivity to MK-801 challenges, and deficits in cognitive flexibility observed in the adult male offspring. Although sometimes contrary to our hypotheses, the overall pattern of our results suggest that prenatal NMDA receptor blockade disrupted normal brain development and induced important behavioural deficits in adulthood.

Keywords : Poly I:C, MK-801, NMDA receptor antagonist, glutamate, dopamine

Acknowledgments

First and foremost, I would like to thank my supervisor, Dr. Uri Shalev, for his constant guidance, support, and trust over the past two years. Furthermore, I would like to express my enormous gratitude to Tracey D'Cunha and Firas Sedki who welcomed me to the lab and provided me with much needed advice and support throughout this process. I also wish to thank Janie Duchesneau who accompanied me during these two years and gave me invaluable moral support and encouragement. I also wish to extend a special thank you to Dan Madularu who generously shared with me his curiosity for schizophrenia and his contagious passion for research. In addition, I want to extend my immense gratitude to Loïc Welch, Maria Athanassiou, Laurie Hamel, and Patricia Martone for their major contributions to this thesis – I could not have accomplished all this work without your help. Of course, I thank all my other current and former labmates for making my time in the lab most enjoyable. I am also grateful to Dr. Wayne Brake and Dr. Dave Mumby for serving on my defence committee.

I would like to express my appreciation to my friends who have been a source of inspiration and support during the past two years, always knowing when to push me to try harder and when to provide me with welcomed distractions: Lisa, Abilash, Kenneth, Nick, and Chema. Finally, I would like to thank my parents, Sylvie and Gerry, and my sisters, Valerie and Cindy, who have given me unconditional love and support that have made this thesis possible.

Table of Contents

List of Figures

General Introduction

Schizophrenia is a psychiatric illness affecting up to 1% of the population (Kulhara & Chakrabarti, 2001; Torrey, 1987). This uniquely human condition has been ranked, in economic terms, the seventh most costly medical illness to modern society (Freedman, 2003) with expenditures reaching approximately $6.85 billion a year in Canada alone (Goeree, O'Brien, & Blackhouse, 2004). Of this total cost estimate, approximately 70% is accounted for by productivity losses associated with morbidity in schizophrenia. Indeed, schizophrenia is characterized by a heterogeneous set of symptoms that make it challenging for those struggling with the disorder to function within societal norms. The clinical onset of these symptoms most commonly occurs during the second or third decade of life and has an average age of diagnosis in males four years earlier than in females (Riecher-Rossler & Hafner, 2000). For research purposes, these symptoms are often grouped into three main categories: positive symptoms (e.g. hallucinations and delusions), negative symptoms (e.g. blunted speech, anhedonia, avolition, etc.), and cognitive symptoms (e.g. impaired working memory, poor cognitive flexibility, etc.). There is yet to be a single theory that fully explains the complex pattern of positive, negative, and cognitive impairment in schizophrenia. Understanding the underlying mechanisms of this disease will shed light into its etiology and will help develop better treatment that will more effectively target the full symptomatology.

Symptoms

Positive symptoms generally reflect an excess of normal functions (Stahl, 2008). Delusions are one type of positive symptoms and refer to misinterpretation of perceptions

or experiences. The content of delusions may follow a variety of themes such as referential (i.e. believing that something refers to oneself), somatic, religious, grandiose, or the most common, persecutory. Hallucinations are another type of positive symptoms and can occur in any sensory modality (e.g. visual, olfactory, gustatory, and tactile) though auditory appears to be the most common. Other symptoms grouped under the positive dimension include disorganized speech and/or behaviour, catatonic behaviour, and agitation. Positive symptoms of schizophrenia are often emphasized as they can be dramatic and surprising. Despite their dramatic nature however, positive symptoms are the symptoms most effectively treated by antipsychotic medication (Stahl, 2008).

Negative symptoms are commonly considered a reduction in normal functions (Stahl, 2008). Without referring to these concepts directly, Emil Kraepelin (1856-1926) was the first to provide a thorough description of what is currently viewed as the negative symptoms of schizophrenia, observing what he called "a core deficit in emotional experience" (Jablensky, 2010). This deficit is associated with the absence of feelings that is responsible for blunted affect, as well as the absence of pleasure from activities, the absence of a desire for activity, and an overall lack of motivation (Azorin, Belzeaux, & Adida, 2014).

Nearly a century later, the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) consensus panel proposed a two-factor model to account for negative symptoms which closely resembles Krapelin's original description: 1) blunted affect-poverty of speech and 2) anhedonia-asociality-avolition (Kirkpatrick & Fischer, 2006). Anhedonia refers to the reduced ability to experience pleasure (e.g. the patient sees previous hobbies or interests as unpleasurable). Ascoiality is a reduced

social drive and interaction (e.g. little sexual interest, little interest in spending time with friends). Avolition refers to a general reduction in desire or motivation (e.g. reduced ability to undertake everyday tasks such as personal hygiene) (Kirkpatrick & Fischer, 2006). The validity of these two subgroups is supported by evidence that distinct groups of patients demonstrating these characteristics are identifiable and clinically distinguishable. For instance, compared with patients from the blunted affect-poverty of speech subgroup, patients in the anhedonia-asociality-avolition subgroup are more likely to have a family history of psychiatric illness, less likely to be employed, more likely to experience poorer premorbid social adjustment, and more likely to experience a gradual onset of psychosis (Strauss et al., 2013).

Negative symptoms in schizophrenia are associated with long periods of hospitalization and poor social functioning (Herbener & Harrow, 2004). Although they may not appear to be as dramatic as the positive symptoms, negative symptoms ultimately determine whether a patient can function well within societal norms. Interestingly, although negative and positive symptoms appear to follow independent courses over time, the negative symptoms are associated with cognitive symptoms (Eaton, Thara, Federman, Melton, & Liang, 1995; van Os & Kapur, 2009).

Eugen Bleuler, who coined the term "schizophrenia" in the early 1900s, argued for impaired cognition rather than delusions and hallucinations as the fundamental dysfunction in the illness (Heinrichs, 2005). Although previous research on schizophrenia has often addressed the positive symptoms, the cognitive symptoms have been described as being the most ubiquitous features of schizophrenia (Bowie & Harvey, 2005) and serving as the prominent determinants of functional disability and poor

prognosis that is observed in a majority of chronically ill patients with schizophrenia (Green, Kern, Braff, & Mintz, 2000). In addition, although antipsychotic treatment has been helpful in relieving positive symptoms and attenuating, to a lesser extent, negative symptoms, cognitive symptoms of schizophrenia remain largely resistant to currently available medications (P. D. Harvey & Keefe, 2001; Keefe, 2007).

Executive functions (e.g. attention, cognitive flexibility) and memory (visual and spatial), both of which are associated with the frontal and medial temporal regions of the brain, are thought to be the cognitive functions most severely affected in schizophrenia(Danielyan & Nasrallah, 2009). Attentional deficits are commonly described as an inability to filter out irrelevant stimuli. There are indications that attentional deficits may constitute a behavioural marker for predisposition to schizophrenia in high risk individuals (e.g. family history of schizophrenia) (Erlenmeyer-Kimling et al., 2000). Sensorimotor gating is a feature of pre-attentive cognitive processing that is consistently reported to be impaired in schizophrenia patients (L. Li, Du, Li, Wu, & Wu, 2009; Swerdlow, Geyer, & Braff, 2001). Its impairments are considered to be an indication of a failure of inhibitory filtering mechanisms that can lead to sensory overload (Geyer, 2006a, 2006b). The most robust correlates of the deficits in sensorimotor gating are abnormalities in distractibility and thought disorder (Geyer, 2006b).

One important operational measure of sensorimotor gating is pre-pulse inhibition (PPI), which has been shown to be impaired in schizophrenia (L. Li et al., 2009). PPI refers to the normal reduction of the amplitude of the startle reflex in response to an intense startling stimulus (the pulse) when this is shortly preceded by a weaker, non-

startling sensory stimulus (the pre-pulse) (L. Li et al., 2009). Another operational measure that is often used to study the neural substrates of complex associative deficits in schizophrenia is latent inhibition. Latent inhibition refers to the instances when an individual learns to ignore a repetitive, trivial stimulus that does not predict an important event. However, when this stimulus is later given meaning as a predictor of an important event, the person must overcome the learned ignore response before new learning can occur (Swerdlow, Braff, Hartston, Perry, & Geyer, 1996). Patients with schizophrenia have been shown to exhibit a deficit in latent inhibition which is reflected as faster learning of the second association in patients with schizophrenia resulting from the inability to gate or suppress the cognitive response to an irrelevant stimulus (Swerdlow et al., 1996).

Impairment in cognitive flexibility is amongst the most reliable deficit observed in schizophrenia (Green & Nuechterlein, 2004). Cognitive flexibility refers to the ability to flexibly change perspectives and to adjust to new demands, rules, or priorities (Diamond, 2013). This is the ability that allows us, for instance, to come up with new ways of tackling problems when previous attempts have failed. It also allows us to adjust our behaviour to meet new demands or take advantage of serendipitous events. One of the most widely used paradigms to assess cognitive flexibility in humans is a set-shifting task known as the Wisconsin Card Sorting Test (WCST) in which the person is asked to match a given choice card with four key cards based on one of the three stimulus dimensions (colour, shape of polygons, number of polygons) (Berg, 1948). After a certain criterion has been reached, the person must discard the old sorting rule and attend to a new one (e.g. switching from a colour-based sorting to shape-based sorting).

Patients with schizophrenia display great difficulty in such tasks that require them to shift between different rules or strategies (Green & Nuechterlein, 2004) . These impairments appear to be due in part to an inability to shift attentional set from one stimulus dimension to another (Floresco, Zhang, & Enomoto, 2009). More specifically, patient's set-shifting deficit is thought to represent a failure to shift from previously rewarded behaviour in response to negative feedback. These impairments have long been attributed to perturbation in frontal lobe functioning (Floresco et al., 2009).

Although Kraepelin and Bleuler both argued that memory functions were relatively preserved in schizophrenia, many studies have since demonstrated that patients with schizophrenia do in fact perform poorly on a wide range of memory tasks (Jablensky, 2010). Recognition memory, the evocation of relevant multimodal memories that enable the experience of familiarity with a given stimulus (Kim et al., 1999), has been shown to be particularly disrupted in schizophrenia. More specifically, schizophrenia patients show deficits in verbal and facial recognition. Amongst the various forms of visual stimuli, faces are especially crucial to humans as their recognition is essential to human social life (Kim et al., 1999). Thus, although such deficits are often viewed as cognitive in nature, they may also overlap with negative symptoms such as asociality.

Neurotransmission in schizophrenia

Alterations in several neurotransmitter systems have been implicated in the pathophysiology of schizophrenia. There are consistent pharmacological and functional data for the involvement of dopamine (DA) and glutamate, giving rise to two main hypotheses that link disruptions of these systems to the neuropathology of schizophrenia.

It should be noted that there is also ample evidence for the involvement of gamma-Aminobutyric acid (GABA), cholinergic, and opioid systems in schizophrenia, but the focus of the present thesis will be on the DA and glutamate systems.

The dopamine hypothesis. One neurochemical hypothesis which has shown remarkable tenacity is the DA hypothesis. DA is a neurotransmitter with highly diversified functions in the brain. As seen in Figure 1, the mesocorticolimbic DA system consists of neurons with cell bodies located in the ventral tegmental area (VTA) projecting to the prefrontal cortex (PFC; mesocortical) and nucleus accumbens (NAcc; mesolimbic) (Stahl, 2008). The classical DA hypothesis of schizophrenia posited that hyperactivity of the DA system was responsible for the symptoms observed in schizophrenia (Carlsson & Lindqvist, 1963). This hypothesis has since been expanded to include the idea that it is the hyperactivity of the mesolimbic DA system that contributes to the positive symptoms in schizophrenia while the hypoactivity of the mesocortical DA system contributes to the cognitive symptoms (Davis, Kahn, Ko, & Davidson, 1991).

The DA hypothesis originally derived from the observation that the antipsychotics used to treat the positive symptoms of schizophrenia act primarily as DA receptor antagonists (Seeman, Chau-Wong, Tedesco, & Wong, 1975; Seeman & Lee, 1975). In addition, high doses of stimulant drugs such as cocaine in healthy individuals can evoke transient psychotic states that resemble positive symptoms of schizophrenia (Lieberman, Kane, & Alvir, 1987). Furthermore, pharmacological studies have demonstrated that exposure to amphetamine (AMPH), a stimulant drug that increases extracellular levels of DA in the striatal regions via release and reverse transport, can exacerbate positive symptoms in schizophrenia patients at doses that do not induce psychosis in healthy

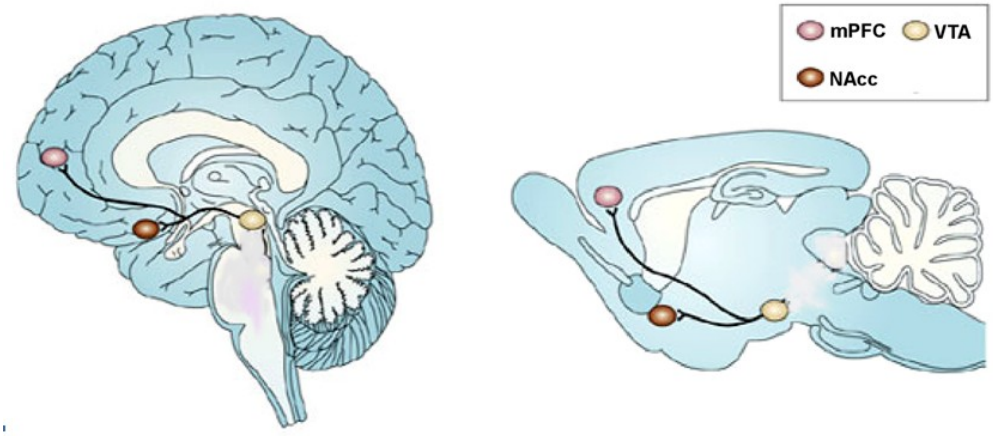


Figure 1. Sagittal view of the human (left) and rat (right) brains, showing the mesolimbic and mesocortical DA pathways, which originate in the ventral tegmental area (VTA) and send ascending projections to the nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC), respectively. Modified from (Laviolette & van der Kooy, 2004).

individuals (Lieberman et al., 1987). More support for the DA hypothesis of schizophrenia comes from imaging studies that have demonstrated marked elevations of AMPH-induced striatal DA release in non-medicated schizophrenia patients compared to healthy volunteers (Abi-Dargham et al., 2000; Laruelle et al., 2000; Laruelle et al., 1996). In addition, analysis of post-mortem brains of patients with schizophrenia have shown increased striatal density of DA D2 receptors in comparison to healthy controls (Seeman et al., 1987). Due to the observation that antipsychotic treatment up-regulate D2 receptors (Soares & Innis, 1999), it is often argued that this finding is a consequence of pharmacotherapy. However, increased D2 levels have also been found in non-medicated patients (Toru et al., 1988).

Hyperactive mesolimbic DA function in schizophrenia is commonly associated with a hypofunctioning mesocortical DA system. Indeed, patients with schizophrenia have been shown to struggle with cognitive tasks that are PFC-dependent such as executive functions and memory. In fact, a study combining imaging techniques and the WCST (associated with PFC functioning) showed that patients had less WCST-related activation in the PFC and that the degree of abnormal activation in the PFC served as a predictor of the magnitude of the mesolimbic DA hyperactivity (Meyer-Lindenberg et al., 2002). Furthermore, decreased DA innervation to the PFC has also been observed in patients with schizophrenia (Abi-Dargham et al., 2000).

The glutamate hypothesis. Glutamate is an amino acid serving as the major excitatory neurotransmitter in the brain and is thought to be dysfunctional in schizophrenia. The postsynaptic effects of glutamate are mediated by three families of gated ion channels: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA),

N-methyl-D-aspartate (NMDA) and kainate (Coyle, 2006; Platt, 2007). There is cumulating evidence that the glutamate dysfunction observed in schizophrenia is mediated by NMDA receptor hypofunction (Duncan, Sheitman, & Lieberman, 1999).

As was the case for the role of DA in schizophrenia, the role of NMDA receptors was determined as a result of pharmacological discovery. Glutamate was first considered as a potential player in schizophrenia in the 1950s when phencyclidine (PCP), an NMDA receptor antagonist, was observed to induce a schizophrenia-like psychotic state in humans (Gordon, 2010; Kantrowitz & Javitt, 2010; Luby, Cohen, Rosenbaum, Gottlieb, & Kelley, 1959). The link to NMDA receptors, however, was not suspected until the 1980s when researchers provided evidence implicating that blockade of the NMDA receptor was the primary mechanism by which PCP produced its effects (D. Lodge, Anis, & Burton, 1982). Subsequent research demonstrated that ketamine and MK-801, NMDA receptor antagonists, also induced psychosis and cognitive deficits closely resembling symptoms of schizophrenia (Coyle, 2006). Furthermore, individuals with schizophrenia show greater sensitivity to the psychomimetic effects of these drugs at doses that have little or no effect in healthy individuals (Javitt & Zukin, 1991). Corroborating the role of glutamate dysfunction in schizophrenia, and more specifically NMDA receptor hypofunction, are findings suggesting that symptoms, particularly the negative symptoms, can be relieved with NMDA receptor modulators such as D-serine, glycine and sarcosine which serve to increase NMDA receptor function (Ross, Margolis, Reading, Pletnikov, & Coyle, 2006). In addition, imaging studies have provided in vivo evidence for reduced NMDA receptor binding in the hippocampus of non-medicated patients with schizophrenia compared to healthy controls (Bressan & Pilowsky, 2000).

This evidence suggests that hypofunction of the NMDA receptor, possibly on critical GABA interneurons, may contribute to the pathophysiology of schizophrenia (Coyle, 2006).

Given the previously mentioned observation that current antipsychotic medications acting primarily through DA D2 receptor blockade fail to relieve all symptoms of schizophrenia, researchers have concluded that these symptoms likely involve neurotransmitter systems other than DA, such as glutamate and GABA. However, this view and the DA hypothesis should not be considered as mutually exclusive. Rather, results point to important interactions between DA receptors and NMDA receptors in critical brain regions such as the hippocampus and between glutamatetamatergic afferents and subcoritcal dopaminergic nuclei (Adler, Freedman, Ross, Olincy, & Waldo, 1999; Breier et al., 1998). Thus, schizophrenia is now believed to involve at least both glutamatetamatergic and dopaminergic dysfunction. One of the greatest challenges for schizophrenia research at present is to understand the etiology of such dysfunctions.

Neurodevelopmental Hypothesis

Many hypotheses have been put forth to explain the etiology of schizophrenia. Of these, one the most widely acknowledged is the neurodevelopmental hypothesis, the view that schizophrenia is the outcome of an aberration in neurodevelopmental processes beginning long before onset of clinical symptoms (Fatemi & Folsom, 2009). Critical processes that are altered include neurogenesis, cell migration, and neuronal connectivity (Ehninger & Kempermann, 2008; Fatemi & Folsom, 2009).

There are several independent lines of evidence that support the conceptualization of schizophrenia as a neurodevelopmental psychiatric disease. For instance, there is no evidence of gross physical damage or signs of progressive neurodegernation and/or ongoing brain inflammatory processes in patients with schizophrenia (Rapoport, Addington, & Frangou, 2005; Weinberger, 1987). In addition, many indicators of congenital anomalies, such as the presence of low-set ears, epicanthal eye folds, and wide spaces between the first and second toes, are present in patients with schizophrenia and indicative of first trimester anomalies (Danielyan & Nasrallah, 2009). In addition to physical manifestations, pre-schizophrenic children present behavioural alterations before the clinical onset of psychosis. These premorbid signs include 'soft' neurological symptoms such as slight posturing of hands and transient choreoathetoid movements. In addition, delays in motor and speech development, poor peer relationships, social isolation, social anxiety, and lower cognitive abilities have been reported to occur more frequently in high-risk children with a schizophrenic parent (Danielyan & Nasrallah, 2009). Importantly, these alterations are present before the clinical onset of schizophrenia and before pharmacological treatment, indicating that they are not the result of progressive degenerative processes or pharmacotherapy (Danielyan & Nasrallah, 2009). Another important observation supporting the neurodevelopmental hypothesis is the observation that individuals who go on to develop schizophrenia are more likely to have experienced pre- or peri-natal adverse events compared to healthy individuals (Fatemi & Folsom, 2009; Weinberger, 1987).

Interaction of genetic and environmental factors

According to the neurodevelopmental hypothesis, the etiology of schizophrenia involves both genetic and environmental factors resulting in disruption of developmental processes occurring as early on as in prenatal development (Fatemi & Folsom, 2009). The genetic component of the gene-environment interaction is supported by a large body of evidence (Danielyan & Nasrallah, 2009; Sawa, Pletnikov, & Kamiya, 2004; Tsuang, 2001). According to twin and adoption studies, more than half of the vulnerability for schizophrenia is of genetic origin (Tsuang, 2001). Although the risk of schizophrenia in the general population is only of approximately 1%, this risk increases substantially in relatives of individuals with the disorder. For instance, monozygotic twins (i.e. sharing 100% of DNA) have a risk of 40-50% when one twin has schizophrenia, whereas this risk decreases to 9% for a dizygotic twin or sibling of an individual with schizophrenia (i.e. sharing approximately 50% of DNA) (Sawa et al., 2004; Tsuang, 2001). Despite the large contribution of genetic factors to schizophrenia, its genetic constituent is considered to be composed of multiples genes with relatively low individual risk factors. For instance, up to 50 candidate genes that may play a role in explaining individual differences in vulnerability or resilience to the development of schizophrenia have been identified (Shirts & Nimgaonkar, 2004). Remarkably, these genes have been shown to play a role in brain development, thereby supporting the role of alterations in neurodevelopment in the etiology of schizophrenia (Harrison & Weinberger, 2005). Nonetheless, the less than perfect monozygotic twin concordance rate for schizophrenia (approximately 50%) indicates the presence of non-genetic contributing factors (Sawa et al., 2004; Tsuang, 2001).

Several environmental risk factors for schizophrenia have been documented. Prenatal events such as maternal stress, famine, substance abuse, infection, or exposure to teratogens during pregnancy have been associated with a higher risk for schizophrenia in adult offspring (Caspi & Moffitt, 2006). Other environmental factors include low birth weight and birth complications as well as postnatal events such as early postnatal trauma, parental negligence during infancy, childhood abuse, premature parental loss, family conflict and violence, substance abuse during adolescence, and head injury (Arndt, Stodgell, & Rodier, 2005; Caspi & Moffitt, 2006). It is, however, important to keep in mind that most of the mentioned environmental risk factors constitute a nonspecific risk for many disorders. For instance, birth complications have been found to be linked to ADHD, mental retardation, and autism spectrum disorders in addition to schizophrenia (Caspi & Moffitt, 2006). Thus, such factors cannot be considered predictive of a particular neurodvelopmental psychiatric disease. Another important consideration is that environmental risk factors are regarded as contributory as exposure to them does not always result in psychiatric illness.

Nonetheless, the evidence from genetic studies and epidemiological research strongly suggests that a gene-environment interaction is involved in the etiology of schizophrenia. The gene-environment interaction implies that in any given population, individual genetic predisposition is directly responsible for the vulnerability to the environmental risk factors of schizophrenia. Human studies have been useful in furthering our understanding of the effects of this interaction on the developing brain. It is, however, impossible to experimentally assess the relative magnitude of the inherited and environmental contributors without the use of animal models (Boksa, 2004).

Neurodevelopmental animal models of schizophrenia

The role of dopamine and glutamate in schizophrenia that is presumed from human studies has received a substantial amount of support from research with animal models (Boksa, 2004, 2010). In line with what has been observed in humans, acute and repeated treatment with dopamine agonists such as cocaine and amphetamine in adult rodents induces psychomimetic responses such as hyperactivity, impairments in sensorimotor gating, working memory, object recognition, and set-shifting. Research aimed at elucidating the role of glutamate has demonstrated that acute and repeated exposure to NMDA receptor antagonists such as PCP, ketamine, and MK-801 in adult rats induces hyperlocomotion, PPI deficits, social deficits, and memory and set-shifting deficits (Neill et al., 2010; Neill, Harte, Haddad, Lydall, & Dwyer, 2014). Results from such animal models have been invaluable in providing further support to the DA and glutamate hypotheses of schizophrenia and in elucidating how these two systems interact with each other. However, these models fail to capture the neurodvelopmental nature of schizophrenia. Neurodevelopmental animal models are based on experimentally induced disruption of brain development that becomes evident in an adult animal in the form of altered neurochemistry and aberrant behaviour and test whether the effects of early brain damage could remain inconspicuous until after a considerable delay as appears to be the case in the human condition (Myslobodsky & Weiner, 2000). There are currently two major lines of neurodevelopmental animal models.

Rather than attempting to reproduce specific putative causative factors implicated in schizophrenia, the first line of models simply attempt to mimic molecular, cellular, and/or systemic aberrations that presumably follow a disruption of early development

analogous to what has been described in human studies (Myslobodsky & Weiner, 2000). This can be accomplished, for example, via lesions or in utero exposure to mitotic toxins. The neonatal hippocampal lesion model, for instance, attempts to reproduce a putative defect in schizophrenia by lesioning of the ventral hippocampus in neonatal rodents (Lipska, Aultman, Verma, Weinberger, & Moghaddam, 2002; Tseng, Chambers, & Lipska, 2009). The absence of proper hippocampal innervation at a critical neurodevelopmental period likely has long-term consequences on synaptic connectivity and function of neural circuits in the prefrontal cortex (Tseng et al., 2009). Indeed, adult rodents exposed to such a neonatal manipulation have been shown to exhibit enhanced amphetamine-induced locomotion, deficits sensorimotor gating, social interaction, and working memory. The MAM (methylazoxymethanol) model attempts to reproduce micrencephaly that has been noted following prenatal exposure to alcohol, irradiation, and cocaine, by administering MAM to pregnant rats at gestation days 14 or 15 (D. J. Lodge & Grace, 2007). A more than 50% decrease in cortical thickness in the offspring has been observed accompanied by motor hyperactivity and deficits in working memory (D. J. Lodge & Grace, 2007). As models of developmental pathology, these models represent a rather crude technique, but provide important information about the function of restricted brain areas and neural connections between them.

The second line of models attempt to test the plausibility of various etiological hypotheses of schizophrenia including malnutrition, obstetric complications, viral infections, and early postnatal stress. For instance, to investigate the influence of systemic viral and bacterial infection during pregnancy, pregnant rats and mice can be injected with viral mimetic polyriboinosinic-polyribocytidilic acid (poly I:C), and

lipopolysaccharide (LPS), respectively (Boksa, 2010; Meyer & Feldon, 2012). Rather consistently, the offspring of pregnant rats injected with poly I:C or LPS have been shown to display augmented AMPH- and MK-801-induced locomotor activity, deficits in sensorimotor gating and working memory, and increased DA release in the nucleus accumbens (Boksa, 2004, 2010). Expanding on this work, some researchers have begun to explore combinations of environmental and/or genetic factors in a "two-hit" hypothesis of schizophrenia. This two-hit hypothesis posits that a first hit, such as genetic vulnerability or an environmental challenge (e.g. prenatal infection) produces a vulnerability, whereas a second hit at a later point in development induces the onset of the illness (Maynard, Sikich, Lieberman, & LaMantia, 2001). For instance, researchers have shown evidence for a synergistic effect of prenatal poly I:C and pubertal cannabinoid exposure on the development of the serotoninergic system in the hippocampus (Dalton, Verdurand, Walker, Hodgson, & Zavitsanou, 2012)

Aim of thesis

Given the apparent robustness of the poly I:C model of maternal infection, our aim in Chapter 1 was to replicate these findings and establish this model in our laboratory with the long-term goals of exploring 1) the common neuromechanisms underlying schizophrenia and substance abuse, and 2) exploring the effects of substance abuse in adolescent rats that have been prenatally exposed to infection, thus testing a two-hit hypothesis of schizophrenia. Inspired by the NMDA receptor hypoactivity hypothesis of schizophrenia, our goal in Chapter 2 was to develop a novel animal model of schizophrenia-like behaviours involving prenatal exposure to the NMDA receptor antagonist, MK-801. More specifically, the goal was to assess the effectiveness of this

model by examining whether it could model key deficits observed in other models of schizophrenia-like behaviours (e.g. drug-induce hyperactivity, memory deficits) as well as behaviours that are thought to model symptoms of schizophrenia that are resistant to current antipsychotic medications (e.g. deficits in cognitive flexibility, social interaction, etc.), and likely under the control of neurotransmitter systems other than dopamine. Moreover, in both chapters, we sought to examine whether these deficits are manifested in adult, but not juvenile rats, as appears to be the case in the human condition.

CHAPTER 1

BEHAVIOURAL CONSEQUENCES OF PRENATAL IMMUNE CHALLENGE IN THE JUVENILE AND ADULT RAT OFFSPRING

Stephanie Gallant, Maria Athanassiou, Laurie Hamel, Uri Shalev

Introduction

There is a substantial amount of epidemiological evidence supporting an association between maternal infection and schizophrenia (Fatemi & Folsom, 2009; Machon, Mednick, & Schulsinger, 1983; Watson, 1984). The first indirect evidence for this association came from the observation of a higher prevalence of schizophrenia in individuals born to winter pregnancies, possibly due to higher prevalence of respiratory infections during this season (Watson, 1984). A higher prevalence rate for schizophrenia has also been found in the offspring of women who were pregnant during influenza epidemics (Machon et al., 1983). In support of this, researchers have indicated significant associations between schizophrenia in the offspring and maternal serologically-documented infections, including rubella, herpes simplex-virus-2, and maternal bacterial infections (A. S. Brown & Derkits, 2010). Given the substantial amount of evidence for this association, a large body of research in developmental psychiatry has been aimed at examining the effects of maternal infection during pregnancy and its effects on the developing fetal brain. Human studies have been useful in furthering our understanding of the effects of maternal infection on the development of the brain. However, the empirical study of the effects of maternal infection on the development of the brain in humans is constrained by many ethical and practical difficulties. Thus, researchers have turned to the use of animal models.

Researchers have used rodents to investigate the consequences of maternal exposure to a variety of immunogenic agents on offspring brain development and behaviour (Boksa, 2010; Meyer & Feldon, 2012). One important behavioural paradigm commonly used in these studies is prepulse inhibition (PPI) of the acoustic startle

response. The acoustic startle response is a full body skeletomuscular response to sudden, and loud acoustic stimuli which can be modulated by the presentation of a weak acoustic prepulse shortly before the startling stimulus (Wolff & Bilkey, 2010). PPI reflects a preattentive sensorimotor gating mechanism which acts to preserve the processing of the prepulse from distracting stimuli such as the startle stimulus. The ability to demonstrate a deficit in PPI is therefore considered to be a hallmark feature of animal models of schizophrenia. Other behavioural paradigms commonly employed in maternal immune activation studies include sensitivity to the locomotor-activating effects of psychostimulant drugs (to assess for mesolimbic DA hyperactivity), and novel object preference (to assess for deficits in recognition memory) (Boksa, 2010).

Initial approaches to examine the effects of maternal infection on offspring brain development and behaviour used prenatal infection with influenza virus followed by the application of a battery of behavioural tests, relevant to various deficits associated with schizophrenia, in the adult offspring (Shi, Fatemi, Sidwell, & Patterson, 2003). Results from these studies showed that adult offspring born to infected dams presented decreased social interaction, reduced exploration in the open field (index of anxiety), impaired performance in the novel object preference task, and impaired PPI of the acoustic startle response (Shi et al., 2003). Subsequent studies investigated the consequences of maternal infection using molecular immunogens. The viral mimic, polyriboinosinic-polyribocytidilic acid (poly I:C), has been used to stimulate the maternal immune system at several stages of pregnancy in mice or rats, ranging from GD 9 until GD 17 (Boksa, 2010; Meyer & Feldon, 2010).

Prenatal challenge with poly I:C induces deficits in PPI of the acoustic startle response and in latent inhibition of conditioned learning (a task measuring associative learning and selective attention) (Meyer & Feldon, 2012). In addition, these offspring show decreased social interaction, impaired novel object recognition, and impairments in spatial working memory (Meyer, Feldon, Schedlowski, & Yee, 2005; Meyer, Nyffeler, Yee, Knuesel, & Feldon, 2008; Shi et al., 2003; Vuillermot, Weber, Feldon, & Meyer, 2010; Wolff & Bilkey, 2008; Zuckerman & Weiner, 2005). These behavioural alterations are thought to model some of the negative and cognitive symptoms of schizophrenia. Prenatal exposure to poly I:C has also been reported to evoke increased sensitivity to the locomotor activating effects of MK-801 and amphetamine (Meyer et al., 2005; Ozawa et al., 2006; Smith, Li, Garbett, Mirnics, & Patterson, 2007; Vuillermot et al., 2010; Zuckerman & Weiner, 2005). Importantly, some of the alterations in behaviour have been shown to appear in the adult but not in juvenile offspring (Ozawa et al., 2006; Romero, Guaza, Castellano, & Borrell, 2008; Vuillermot et al., 2010; Zuckerman & Weiner, 2003), as occurs in schizophrenia. Also of significance is the observation that a number of behavioural alterations have been shown to be reversed by either acute or chronic treatment with several antipsychotic drugs (Meyer, Nyffeler, Yee, et al., 2008; Ozawa et al., 2006; Romero, Guaza, Castellano, & Borrell, 2010; Shi et al., 2003; Zuckerman & Weiner, 2005).

Given the central role of DA in schizophrenia, several studies have investigated the effects of prenatal poly I:C on this neurotransmitter system. One common approach is the measurement of tissue DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). These metabolites are the product of the

enzymatic degradation of DA by the monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). HVA/DA or DOPAC/DA ratios are often used as a measure of the turnover of this neurotransmitter. Prenatal poly I:C treatment (i.v., 4 mg/kg) at GD 15 resulted in enhanced release of DA in the striatum in adult offspring (Zuckerman, Rehavi, Nachman, & Weiner, 2003). Poly I:C treatment (i.v., 5 mg/kg) at GD 9 has been reported to increase DA and its major metabolites DOPAC and HVA levels in the PFC in the adult offspring (Winter et al., 2009). Along the same lines, repeated injections of poly I:C (i.p., 5 mg/kg, GD 12-17) increased levels of DOPAC and HVA in the adult striatum (Ozawa et al., 2006).

Several other structural and neurochemical alterations that parallel the pathophysiological features of schizophrenia have been reported in the offspring of rodents born to poly I:C-treated dams. For instance, prenatal poly I:C has been shown to result in enlarged lateral ventricles which are commonly reported in post-mortem brains of patients with schizophrenia (L. Li et al., 2009). In addition, there have been reports of decreased hippocampal neurogenesis (Meyer, Schwendener, Feldon, & Yee, 2006) as well as decreased dendritic arborisation, especially in the CA1 (Baharnoori, Brake, & Srivastava, 2009). There are also reports of alterations in GABA and glutamate neurotransmitter systems in the hippocampal formation (Meyer et al., 2006; Winter et al., 2009).

Collectively, the effects of prenatal poly I:C in the offspring are consistent with the epidemiological link between maternal infection and schizophrenia in humans. Moreover, these results suggest that immune activation during gestation via poly I:C treatment can induce changes in neurodevelopment that result in robust alteration of brain

function and behaviour in the adult offspring. The poly I:C model of schizophrenia-like symptoms thus appears to be a reasonable place to begin exploring the combined consequences of prenatal and adolescent environmental challenge. The first step in such an endeavour is, of course, to replicate previous findings with the poly I:C model. Therefore, the overarching goal of the work presented in this first chapter was to replicate the findings that prenatal exposure to poly I:C would result in altered behaviour and neurochemistry in the adult offspring. We hypothesized that adult rats born to poly I:C-treated dams would exhibit deficits in object recognition memory, enhanced sensitivity to the locomotor activating effects of amphetamine, and impaired PPI of the startle response. Importantly, we predicted that these deficits would be manifested in adult, but not juvenile offspring.

Materials & Methods

Subjects

Timed pregnant Wistar dams (Gestation day (GD) 7; Charles River Laboratories, Québec, Canada) were individually housed in a colony room on a 12 h light-dark cycle (lights on at 8:00 AM; 21˚C) with *ad libitum* access to food and water. Females were weighed daily in an effort to monitor their pregnancies and habituate them to daily handling. At GD 15, dams were mildly restrained in a towel and given an intravenous injection of either 0.9% saline (n = 4) or 8.0 mg/kg poly I:C via the tail vein (n = 5). Dams were otherwise left undisturbed until the day after parturition. The day of parturition was designated postnatal day (PND) 0. On PND 1, litters were weighed and culled to 10 pups per litter (5 males and 5 females when possible). Litters were left undisturbed until weaning at PND 21 and housed with same-sex offspring of the same

treatment condition. Different sets of rats were tested during the juvenile period (PND 30-40) and adulthood (PND 70+). All behavioural testing was conducted during the light phase of the cycle. All experiments were performed in accordance with the guidelines of the Canadian Council of Animal Care and all animal procedures were approved by the Animal Research Ethics Committee of Concordia University.

Apparatus & Procedure

IL-6 plasma level assessment. Researchers have reported batch and lot variations in the pyrogenic and cytokinogenic activity of commercial poly I:C (L. Harvey & Boksa, 2012). Thus, the dosage for poly I:C was determined during a preliminary experiment as follows. We obtained samples of poly I:C from two independent batches (all from Sigma – Aldrich, Canada). Female non-pregnant rats received an intravenous injection via the tail vein of 4.0 mg/kg poly I:C from batch 1 (n = 2), 4.0 mg/kg poly I:C from batch 2 (n = 2), 8.0 mg/kg poly I:C from batch 1 (n = 2), 8.0 mg/kg poly I:C from batch 2 (n = 2), or 0.9% saline (n = 4) and IL-6 responses were compared. IL-6 is the primary circulating pro-inflammatory cytokine mediating the acute phase inflammatory response (Heinrichs, 2005) and previous studies have shown that, in rats, an intravenous injection of poly I:C at GD 15 results in peak elevations of IL-6 in the dams' blood, 6 h after injection. Thus, blood was collected in ice-cold vials 6 h after injection and immediately centrifuged. Plasma was stored at - 80˚C until being assayed for IL-6 using a commercially available ELISA kit (R&D Systems, Minneapolis, MN). The range of the assay is between 0 and 4000 pg/mL and the reported inter-assay variation is 7-10%.

Maternal behaviour and pup weights. Maternal behaviour of SAL (n = 4) and poly I:C treated dams (n = 5) was observed twice a day each day for the first 14 days following parturition to assess whether the prenatal manipulation resulted in differences in dam behaviour. A rater made observations once per minute in 10-min increments at two different times (09:30 and 16:00 hrs). As previously described (Priebe et al., 2005) maternal behaviours observed included: (1) arched-back nursing; dam is in an arched position over the nursing pups, (2) nursing prone; dam is lying on its side while pups are nursing, (3) grooming/licking, (4) nest building, and (5) off-nest. Maternal behaviours were converted to a percentage of the number of times the dam engaged in each of these behaviors over the total number of time points observed (e.g. 1 observation/min/dam X 10 min/session X 2 sessions/day X 14 days = 280 observations/dam). Pups (Males: SAL, n = 24; poly I:C, n = 15; Females: SAL, n = 22; poly I:C, n = 21) were weighed at PND 1, 8, 14, and 21.

Novel object preference (NOP). An open field arena (60 cm x 70 cm x 70 cm) constructed of gray PVC plastic with a stainless steel flooring covered with wood chip bedding was used. A web camera was positioned above the arena and both the familiarization and test phases were recorded for later analysis. Objects made of glass, porcelain, or glazed ceramic and varying in height between approximately 6 and 12 cm were used as test stimuli. The objects were attached with epoxy to a small glass jar (6 cm high) and two inverted jar lids were attached to the arena floor each at 27 cm from opposing corners of the arena. Objects were fixed in place by screwing the jars into the lids (Figure 2). There were three copies of each object. All objects used have been tested for investigatory preference and matched so that each object type used in the pairs elicits

A

B

Figure 2. (A) Familiarization phase and (B) retention phase of the novel object preference (NOP) task.

a similar amount of investigation. Objects used as the novel stimulus and their position in the box were counterbalanced. Rats were tested during the juvenile period (Males: SAL, *n* = 8; poly I:C, *n* = 6; Females: SAL, *n* = 6; poly I:C, *n* = 9) or in adulthood (Males: SAL, *n* = 7; poly I:C, *n* = 8; Females: SAL, *n* = 6; poly I:C, *n* = 9). The task was divided into three phases: habituation, familiarization, and retention test (Gaskin et al., 2010).

Habituation. Rats were individually placed inside the arena with two identical objects for 10 min on two consecutive days. NOP testing began 24 hrs after the second habituation session.

Familiarization. Rats were placed into the arena containing two copies of the sample object, for 5 min. During the familiarization phase, total exploration time was recorded to ensure that there were no statistically significant differences between treatment conditions and that rats explored for a minimum of 1 min. Rats were returned to their home cages for a delay period. The delays used were 90 sec, 15 min, and 4 hrs.

Retention test. Following the delay, rats were placed inside the arena for 5 min with a copy of the sample object and a novel object. Object investigation was defined as the rat's head facing, touching, and/or sniffing the object at a distance of or less than 2 cm. Climbing or sitting on objects was not considered investigation. Investigation times were recorded using ODLog version 2.7.2 (Macropad, software) and an investigation ratio was computed for the retention test. This investigation ratio consisted of the time spent investigating the novel object relative to total object investigation ($t_{novel}/(t_{novel} + t_{familiar})$) during the first minute of the test because investigation of the novel object subsides as the trial progresses (Dix & Aggleton, 1999).

Drug-induced locomotor activity. Locomotor activity was quantified with an infrared activity-monitoring apparatus for rats (Truscan, Coulbourn Instruments, Whitehall, PA, USA). The apparatus were clear polyethylene test chambers 39 cm X 42 cm X 50 cm with 16 light-emitting diode photodetector pairs located along the base and spaced every 2.5 cm. Data were captured using Trusacan2 software in total distance traveled (successive beam interruptions (cm)). All rats were tested throughout the experiment in the same respective activity chamber at the same time of day.

Juvenile. SAL (Males: *n* = 5; Females: *n* = 6) and poly I:C (Males: *n* = 5; Females: *n* = 8) rats were tested during the juvenile period. On day 1, rats were placed in the box for 30 min prior to injection. Following this habituation period, all rats received an injection of 0.9% saline (i.p.) and were left in the activity chamber for an additional 90 min. On days 2 and 3, rats underwent the same procedure but received an i.p. injection of 0.75 mg/kg and 1.0 mg/kg amphetamine respectively.

Adults. Drug-naive SAL (Males: *n* = 6; Females: *n* = 6) and poly I:C (Males: *n* = 7; Females: *n* = 8) rats were tested during adulthood. Rats were tested under the same protocol and same doses described above for the juvenile period.

Prepulse inhibition of the acoustic startle response. At PND 22, male (SAL: *n* = 5; poly I:C: *n* = 8) and female (SAL: *n* = 6; poly I:C: *n* = 8) drug-naive offspring were transported to the Douglas Mental Health University Institute where they were left undisturbed for an acclimatization period of 10 days prior to testing. All testing occurred over one day. PPI was measured in 4 startle chambers (San Diego Instruments, San Diego, CA) consisting each of a Plexiglas tubes mounted on a Plexiglas base within a sound-attenuating chamber. Chamber assignment was counterbalanced for prenatal

treatment and chambers were cleaned with a 70% alcohol solution between animals. A piezoelectric strain meter attached to the base transduced the startle response and was digitized and recorded by a computer. A speaker located in the ceiling of the sound attenuating chamber presented all acoustic stimuli and maintained a constant background noise level of 70 dB. Startle reactivity was assessed by exposing animals to a 40 ms, 120 dB acoustic stimulus alone. An average of fifty 1-ms readings, beginning at the onset of the startle stimulus, was used as the dependent variable. PPI of acoustic startle responses was measured by having the 120 dB startle stimulus proceeded by a 20 ms prepulse stimulus of 4, 8, 12, and 16 dB above background, which terminated 80 ms before the onset of the startle stimulus (prepulse-pulse trials). The testing session consisted of 12 startle-only trials, 12 no-stimulus (null) trials, and 24 prepulse-pulse trials. Each session began with a 5 min acclimation period of background noise. This was followed by trials arranged in pseudorandom order to prevent consecutive presentations of the same trial type. The degree of PPI of acoustic startle response was calculated as a percentage for each prepulse intensity using the following formula:

100% X (1- (mean prepulse trial – mean null)/(mean startle – mean null))

Drugs. *D*-Amphetamine sulfate (AMPH, Sigma-Aldrich) was dissolved in 0.9% saline. Poly I:C (Sigma-Aldrich) was dissolved in 0.2 mL phosphate buffered saline (PBS).

Statistical Analysis

All statistical analyses were conducted using SPSS version 22 for Windows (IBM, Chicago, IL). All variables of interest were assessed for normality and equality of variance. In all *t*-tests where the Levene's test for equality of variance was violated,

corrected degrees of freedom were used. Likewise, in all Analyes of variance (ANOVAs) where the Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was used. Effect sizes were computed for all tests. For *t*-tests, Cohen's *d* were calculated when sample sizes were equal while Hedge's *g* was calculated for unequal samples sizes (Cohen, 1988; Hedges & Olkin, 1985). Partial eta squared was calculated for all ANOVAs.

Maternal behaviour and pup weights. Independent samples *t*-tests were used to compare SAL and poly I:C-treated dams on each of the maternal behaviours. Treatment condition (SAL vs. Poly I:C) served as the independent variable while percentage frequency of behaviour served as the dependent variable Independent samples *t*-tests were also used to compare body weights of SAL and poly I:C pups at each of the four PNDs with treatment condition serving as the independent variable and body weight serving as the dependent variable.

Novel object preference. A one sample *t*-test was used to compare each group's investigation ratio to chance (0.5). Prenatal treatment (SAL vs. Poly I:C) served as the independent variable while investigation ratio served as the dependent variable. Investigation ratios that were significantly above chance level indicated that rats displayed preference for the novel object, suggesting that rats recognized the familiar object.

Drug-induced locomotor activity. Although it is customary to display and analyze locomotor activity data across time, we were unable to retrieve the activity data across time due to technical difficulties. As a result, activity data in this chapter are presented as total distance traveled during the entire session. Independent samples *t*-tests

were used to compare SAL and poly I:C rats on each testing day. Prenatal treatment served as the independent variable while total distance travelled (cm) served as the dependent variable.

Prepulse inhibition of the acoustic startle response. Independent samples *t*-tests were used to compare SAL and poly I:C rats in null trials and startle-only trials. Prenatal treatment was used as the independent variable while movement was used as the dependent variable. A 2 X 4 mixed ANOVA was used to analyze PPI. *Prenatal treatment* (SAL, poly I:C) served as the between-subject factor while *prepulse intensity* (4, 8, 12, 16) served as the within-subject factor.

Results

Maternal behaviour and pup weights

There were no statistically significant differences between poly I:C- and saline-treated dams on any of the five assessed maternal behaviours (Figure 3). Thus, the treatment condition did not affect the way in which dams cared for their offspring during the first 14 days of life. Body weights of both male and female poly I:C and SAL offspring did not differ significantly at PND 1, 8, 14, and 21 (Figure 4).

Novel object preference

Males

Juvenile. Following a 90 sec delay, the poly I:C rats showed statistically significant preference for the novel object, $t(7) = 3.18$, $p = .015$, $d = 1.12$, while the SAL rats did not, $t(5) = 1.17$, $p = .296$, $d = 0.48$. Following a 15 min delay however, neither the SAL, $t(6) = 1.23$, $p = .266$, $d = 0.46$, or poly I:C rats, $t(6) = 0.90$, $p = .403$, $d = 0.34$, showed statistically significant novelty preference. In addition, no novelty preference

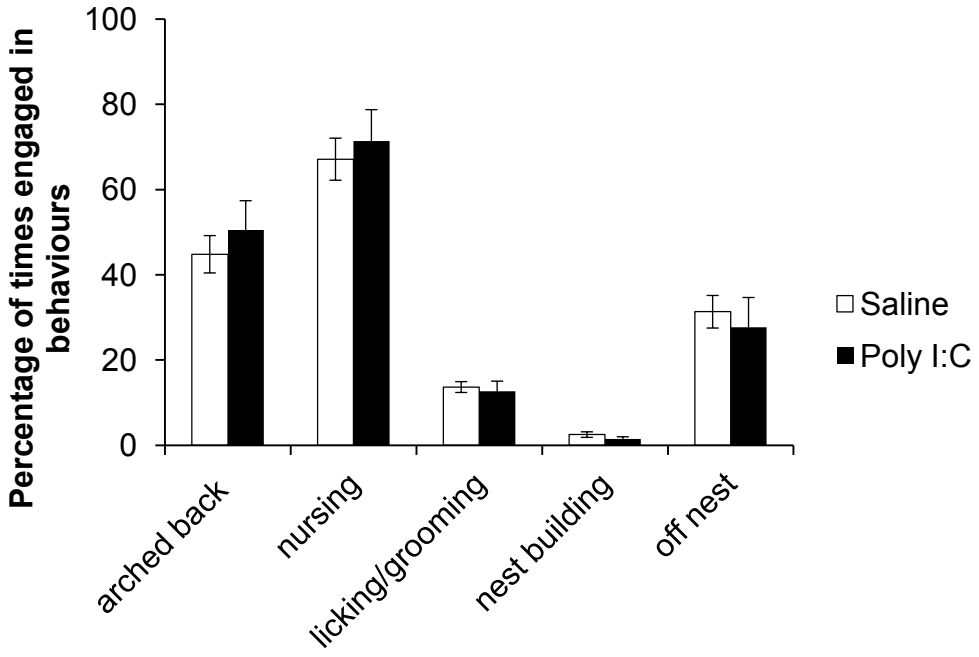


Figure 3. Mean (± SEM) percentage of number of times Saline and poly I:C dams engaged in maternal behaviours. At gestation day 15, dams were given an intravenous injection of either 0.9% saline or 8.0 mg/kg poly I:C via the tail vein. See text for detailed description of maternal behaviours.

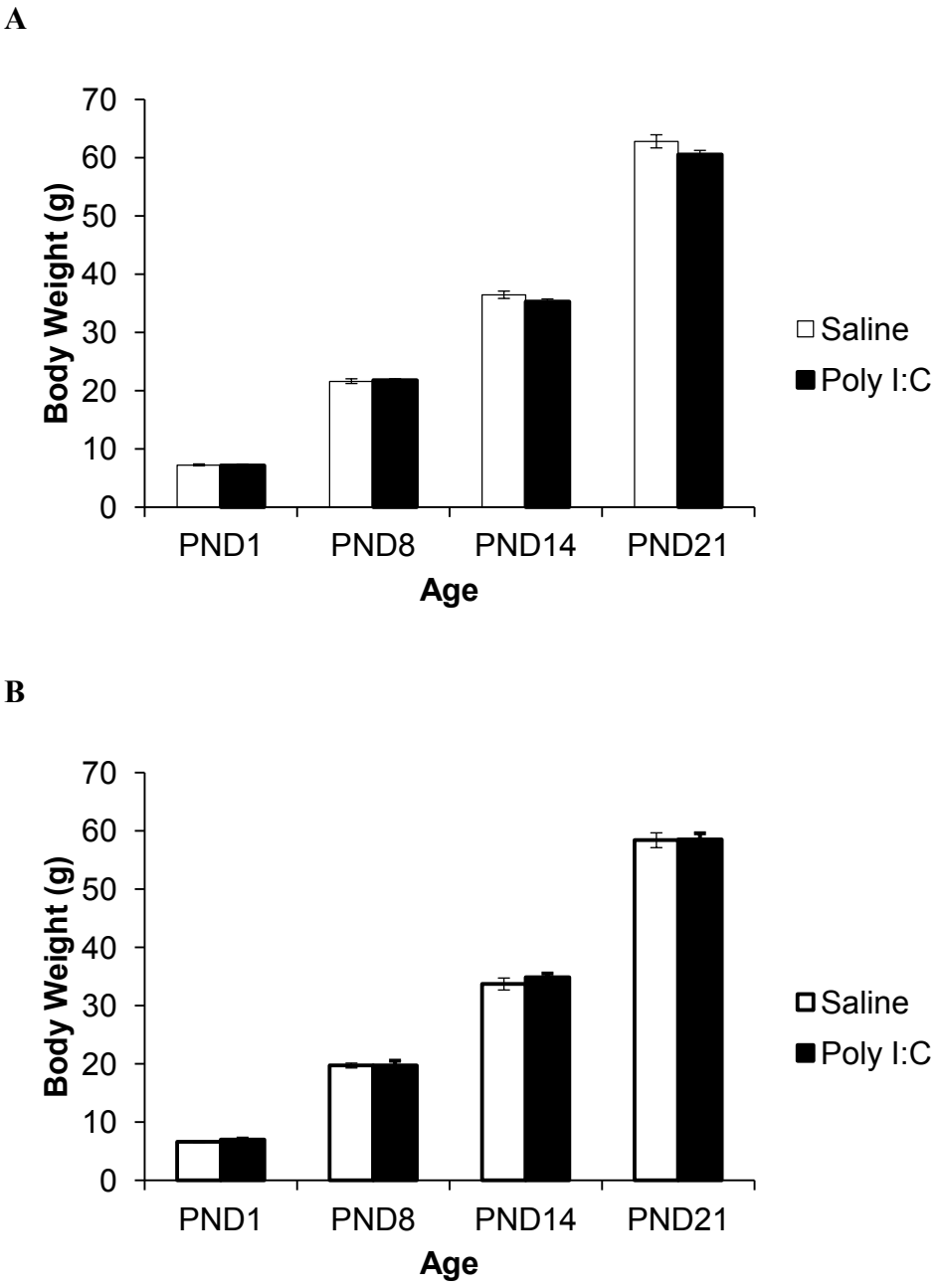


Figure 4. Mean (± SEM) body weights of (A) male and (B) female offspring at postnatal days 1, 8, 14, and 21 in the prenatal poly I:C and Saline rats.

was observed following a 4 h delay in neither the SAL, $t(6) = 0.96$, $p = .374$, $d = 0.36$, or poly I:C rats, $t(8) = 1.40$, $p = .205$, $d = 0.49$ (Figure 5 A).

Adults. As seen in Figure 5 B, following a 90 sec delay both the SAL, $t(6) = 3.50$, $p = .013$, $d = 1.32$, and poly I:C rats, $t(7) = 7.74$, $p = .000$, $d = 2.74$, showed statistically significant novel object preference. After a 15 min delay, both the SAL, $t(6) = 4.01$, $p = .007$, $d = 1.52$, and poly I:C rats, $t(7) = 5.49$, $p = .001$, $d = 1.94$, continued to show significant preference for the novel object. However, following a 4 h delay, neither the SAL, $t(6) = 1.25$, $p = .257$, $d = 0.47$, nor the poly I:C rats, $t(7) = 1.82$, $p = .276$, $d = 0.42$, showed statistically significant novelty preference.

Females

Juvenile. As seen in Figure 6 A, following a 15 min delay, neither the SAL, $t(5) = 0.59$, $p = .581$, $d = 0.24$, nor the poly I:C, $t(8) = 2.03$, $p = .077$, $d = 0.68$, rats showed statistically significant novelty preference. The same was observed following a 4 h delay where neither the SAL, $t(5) = 0.74$, $p = .494$, $d = 0.19$, nor the poly I:C, $t(8) = 1.44$, $p = .187$, $d = 0.45$, rats showed preference for the novel object.

Adults. As seen in figure 6 B, following a 90 sec delay, both the SAL, $t(5) = 10.16$, $p = .000$, $d = 10.16$, and poly I:C rats, $t(8) = 9.24$, $p = .000$, $d = 9.24$, showed statistically significant novelty preference. After a 15 min delay, both the SAL, $t(5) = 3.83$, $p = .012$, $d = 3.83$, and poly I:C rats, $t(8) = 4.77$, $p = .001$, $d = 4.77$, continued to show significant preference for the novel object. However, following a 4 h delay, the SAL rats, $t(5) = 6.17$, $p = .002$, $d = 6.17$, showed preference for the novel object while the poly I:C rats did not, $t(8) = 0.50$, $p = .632$, $d = 0$.

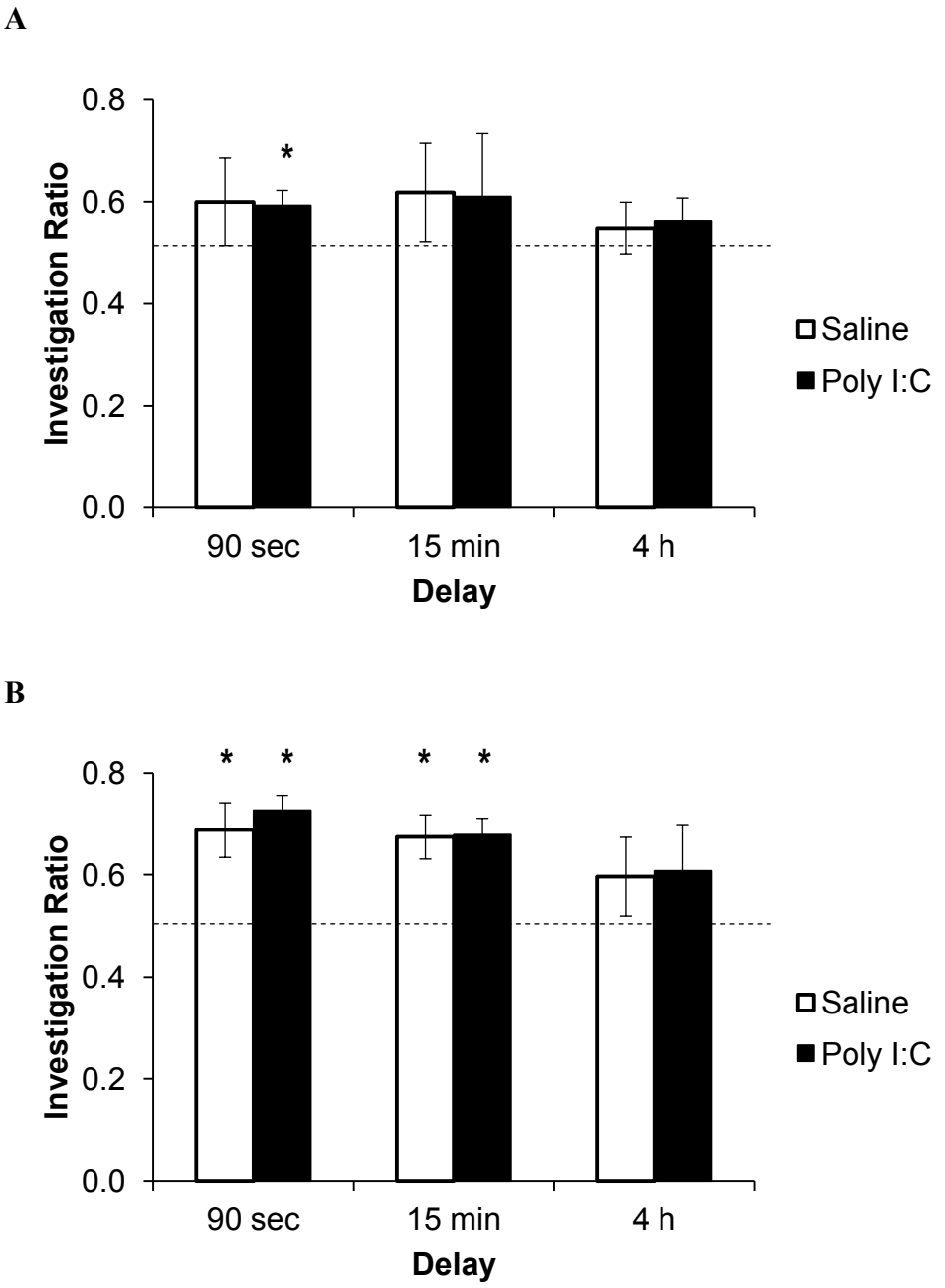


Figure 5. Mean (±SEM) investigation ratio for the first min of the novel object preference test sessions for (A) juvenile prenatal Saline and poly I:C and (B) adult prenatal Saline and poly I:C male offspring. *Significantly different from 0.5, $p < 0.05$.

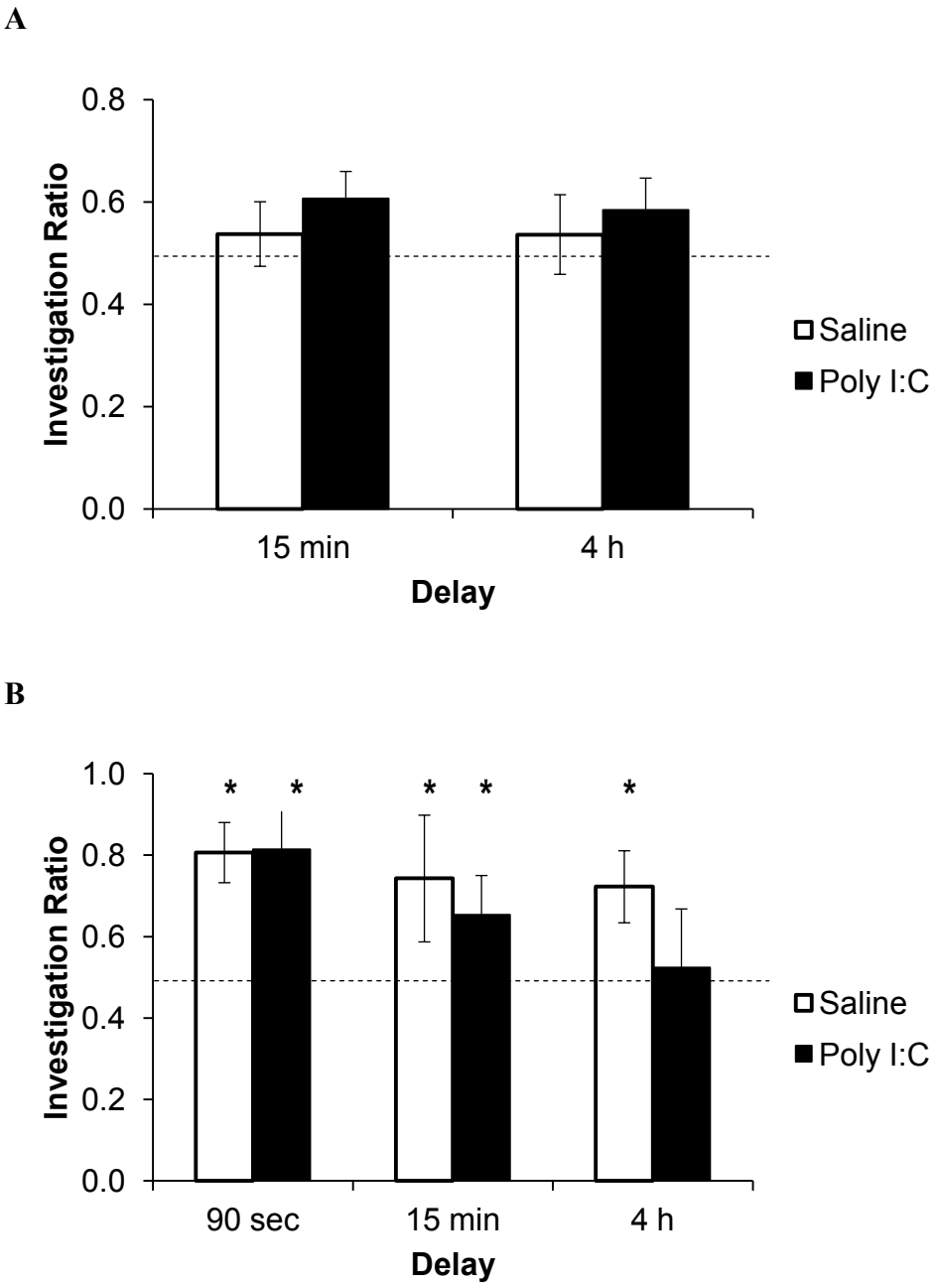


Figure 6. Mean (±SEM) investigation ratio for the first min of the novel object preference test sessions for (A) juvenile Saline and poly I:C and (B) adult Saline poly I:C female offspring. *Significantly different from 0.5, $p < 0.05$.

Amphetamine-induced locomotor activity

Males

Juvenile. As seen in Figure 7 A, following the saline injection, there were no statistically significant differences between SAL and poly I:C rats in total distance travelled, $t(8) = 0.19$, $p = .854$, $g = 0.12$. The total distance travelled observed following a low dose of amphetamine (0.75 mg/kg),appeared to be higher in the poly I:C rats compared to the SAL rats, but the difference was not statistically significant, $t(5.21) = 1.79$, $p = .132$, $g = 1.13$. However, following a high dose of amphetamine (1.0 mg/kg), poly I:C rats' total distance travelled was again higher than in the SAL rats, and this group difference approached statistical significance, $t(8) = 2.11$, $p = .068$, $g = 1.33$.

Adulthood. Following the saline injection, there were no statistically significant differences in total distance travelled between SAL and poly I:C rats, $t(11) = 0.82$, $p = .432$, $g = 0.45$. Similarly, no differences in total distance travelled were observed between SAL and poly I:C rats following a low dose of amphetamine (0.75 mg/kg), $t(11) = 0.187$, $p = .855$, $g = 0.10$, or a high dose of amphetamine (1.0 mg/kg), $t(11) = 0.36$, $p = .726$, $g = 0.20$ (Figure 7 B).

Females

Juvenile. As seen in figure 8 A, there were no differences in total distance travelled between SAL and poly I:C rats following an injection of saline, $t(12) = 0.86$, $p = .407$, $g = 0.49$. Similarly, no differences in total distance travelled were observed between SAL and poly I:C rats following a low dose of amphetamine (0.75 mg/kg), $t(12) = 1.67$, $p = .122$, $g = 0.94$, or a high dose of amphetamine (1.0 mg/kg), $t(12) = 0.28$, $p = .780$, $g = 0.16$.

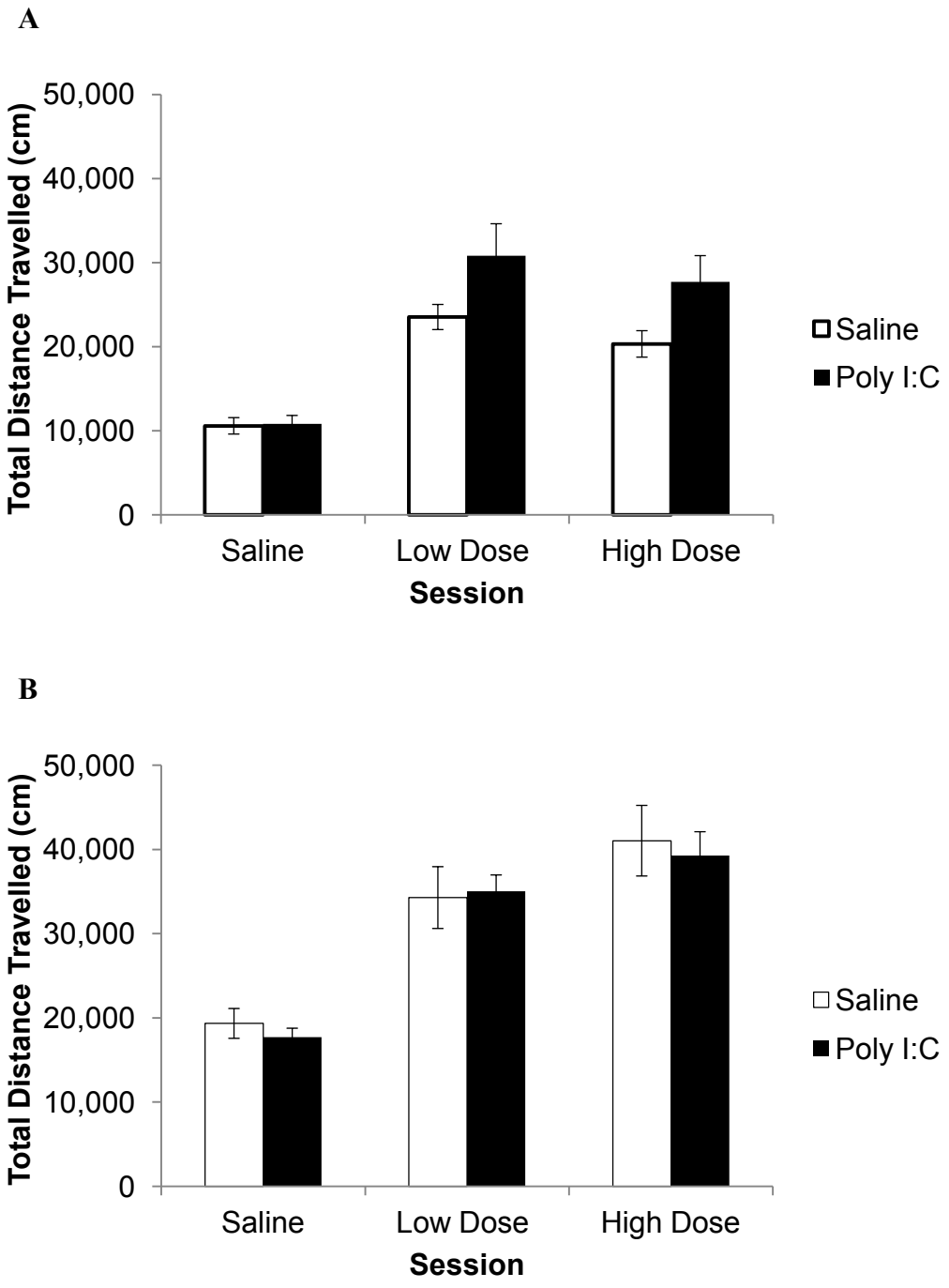
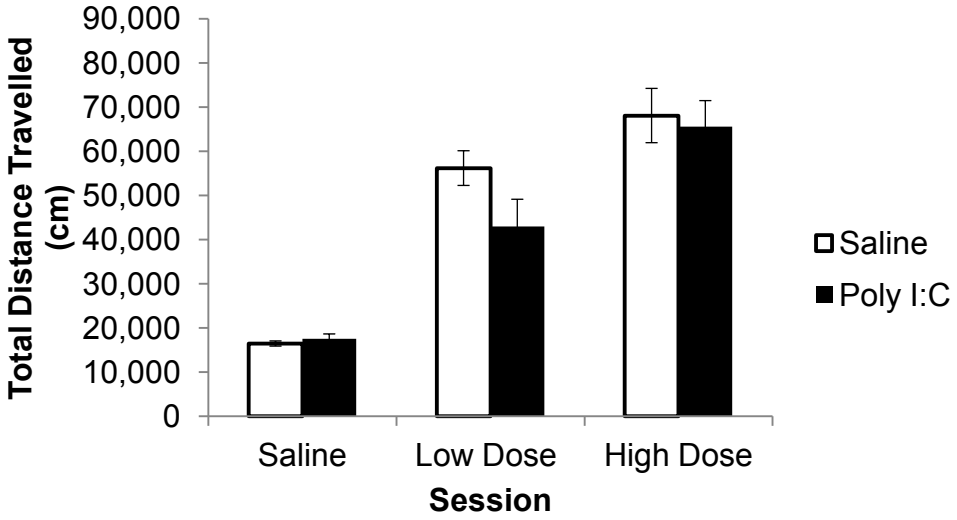


Figure 7. Mean (±SEM) total distance travelled for (A) juvenile Saline and poly I:C and (B) adult Saline and poly I:C male offspring. Locomotor activity was assessed following acute injections of saline or amphetamine (0.75, 1.0 mg/kg; i.p.) before the test session.

A



B

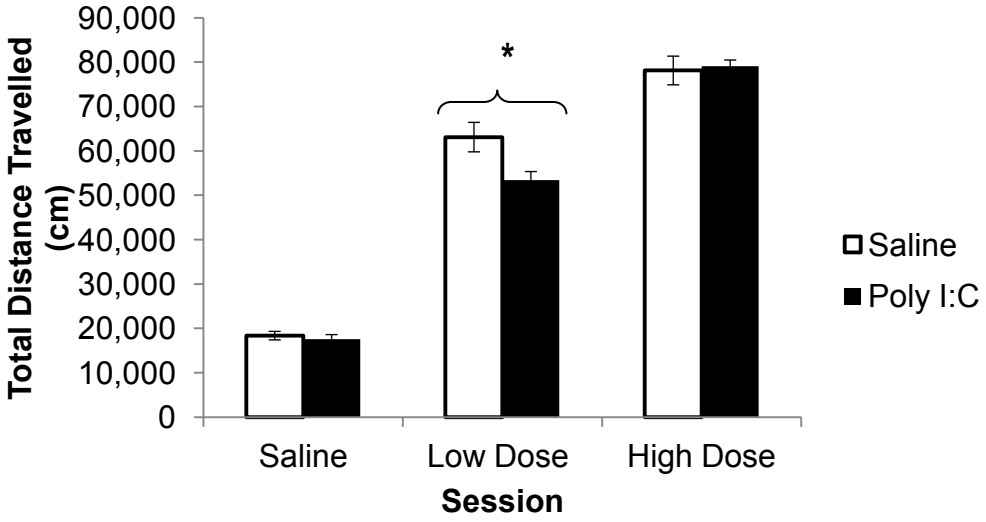


Figure 8. Mean (±SEM) total distance travelled for (A) juvenile Saline and poly I:C and (B) adult Saline and poly I:C female offspring following an amphetamine injection (0.0, 0.75, 1.0 mg/kg; i.p.). *Significantly different, $p < 0.05$. Locomotor activity was assessed following acute injections of saline or amphetamine (0.75, 1.0 mg/kg; i.p.) before the test session

Adulthood. As was observed in juvenile females, there were no differences in total distance travelled between SAL and poly I:C adult females following an injection of saline, $t(12) = 0.55$, $p = .592$, $g = 0.30$ (Figure 8 B). Interestingly, following a low dose of amphetamine (0.75 mg/kg), poly I:C rats moved significantly less than SAL rats, $t(12) = 2.67$, $p = .021$, $g = 1.44$. However, following a high dose of amphetamine (1.0 mg/kg), there were no statistically significant differences between SAL and poly I:C rats in distance travelled, $t(12) = 0.29$, $p = .777$, $g = 0.16$.

Prepulse inhibition of acoustic startle response

Males

Baseline measures. As seen in Figure 9 A, there were no statistically significant differences between SAL and poly I:C rats in the magnitude of activity during the null trials, $t(11) = 0.20$, $p = .848$, $g = 0.11$. Similarly, no statistically significant differences were found between SAL and poly I:C rats in the magnitude of response during the startle-only trials, $t(11) = 0.63$, $p = .544$, $g = 0.36$. The results from these baseline measures indicate that the prenatal treatment did not influence baseline activity within the chamber or general reactivity or sensitivity to the startle pulse. Thus, potential differences observed in prepulse inhibition cannot be attributed to baseline differences.

PPI. As illustrated in figure 9 B, the ANOVA revealed a statistically significant main effect for *prepulse intensity*, $F(3, 33) = 10.66$, $p < .001$, $\eta_p^2 = 0.49$. Simple contrasts indicated that percent PPI increased significantly as the prepulse intensity increased, $F(1, 12) = 22.29$, $p < .001$, $\eta_p^2 = 0.65$, suggesting that louder prepulses were more effective at inhibiting the startle response to the pulse. However, there were no differences between SAL and poly I:C rats in PPI at any of the intensities, *prenatal*

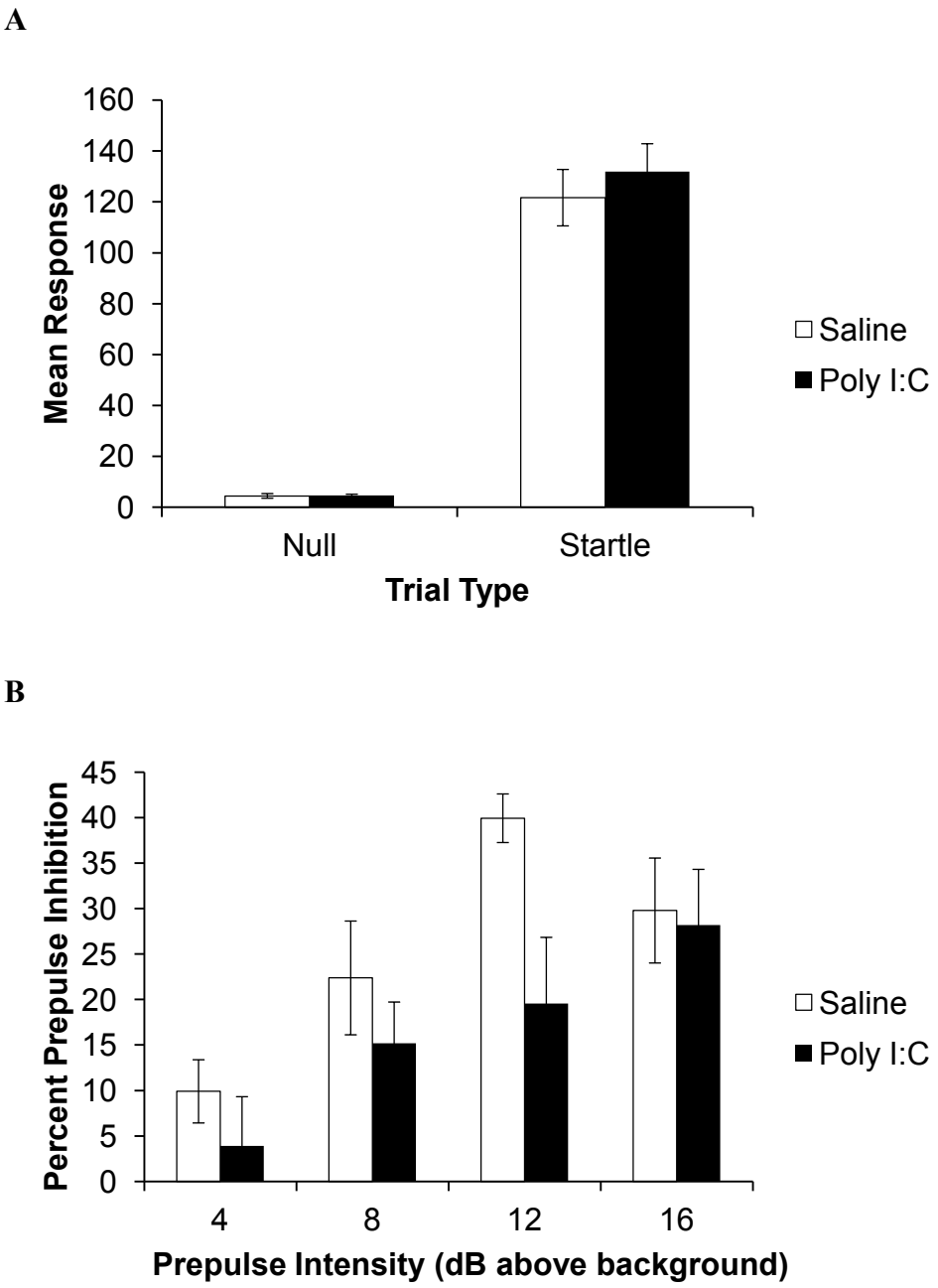


Figure 9. Mean (±SEM) of (A) magnitude of response in null trials and startle-only trials and (B) percent prepulse inhibition of the acoustic startle response in Saline and poly I:C male juvenile rats. *Significantly different, $p < 0.05$.

treatment: F (1, 11) = 1.96, p = .189, $\eta_p^2 = 0.15$. The *prenatal treatment* by *prepulse intensity* interaction was not statistically significant, F (3, 33) = 1.52, p = .227, $\eta_p^2 = 0.12$.

Females

Baseline measures. As was found with male offspring, there were no statistically significant differences between SAL and poly I:C female rats in the magnitude of activity during the null trials, $t(12) = 0.40$, $p = .693$, $g = 0.22$, or in the magnitude of response during the startle-only trials, $t(12) = 0.43$, $p = .672$, $g = 0.23$ (Figure 10 A).

PPI. As was the case for male rats, the ANOVA revealed a statistically significant main effect for *prepulse intensity*, F (3, 26) = 7.40, p < .001, $\eta_p^2 = 0.46$, with simple contrasts again indicating that percent PPI increased significantly as the prepulse intensity increased, F (1, 12) = 22.29, p < .001, $\eta_p^2 = 0.65$ (Figure 10 B). There were no differences between SAL and poly I:C rats in PPI at any of the intensities, prenatal treatment: F (1, 12) = 0.03, p = .870, $\eta_p^2 = 0.002$, and the *prenatal treatment* by *prepulse intensity* interaction was not statistically significant, F (3, 36) = 0.39, p = .760, $\eta_p^2 = 0.03$.

Summary

No differences were found in maternal behaviour between SAL and poly I:C-treated dams suggesting that treatment condition did not impact the way in which dams cared for their offspring during the first 14 days following parturition. It was thus deemed safe to assume that any observed behavioural differences between SAL and poly I:C offspring were not the result of differences in maternal care. Our hypothesis that poly I:C offspring would have lower weights compared to SAL offspring was not supported, with no differences found at PND 1, 8, 14, or 21.

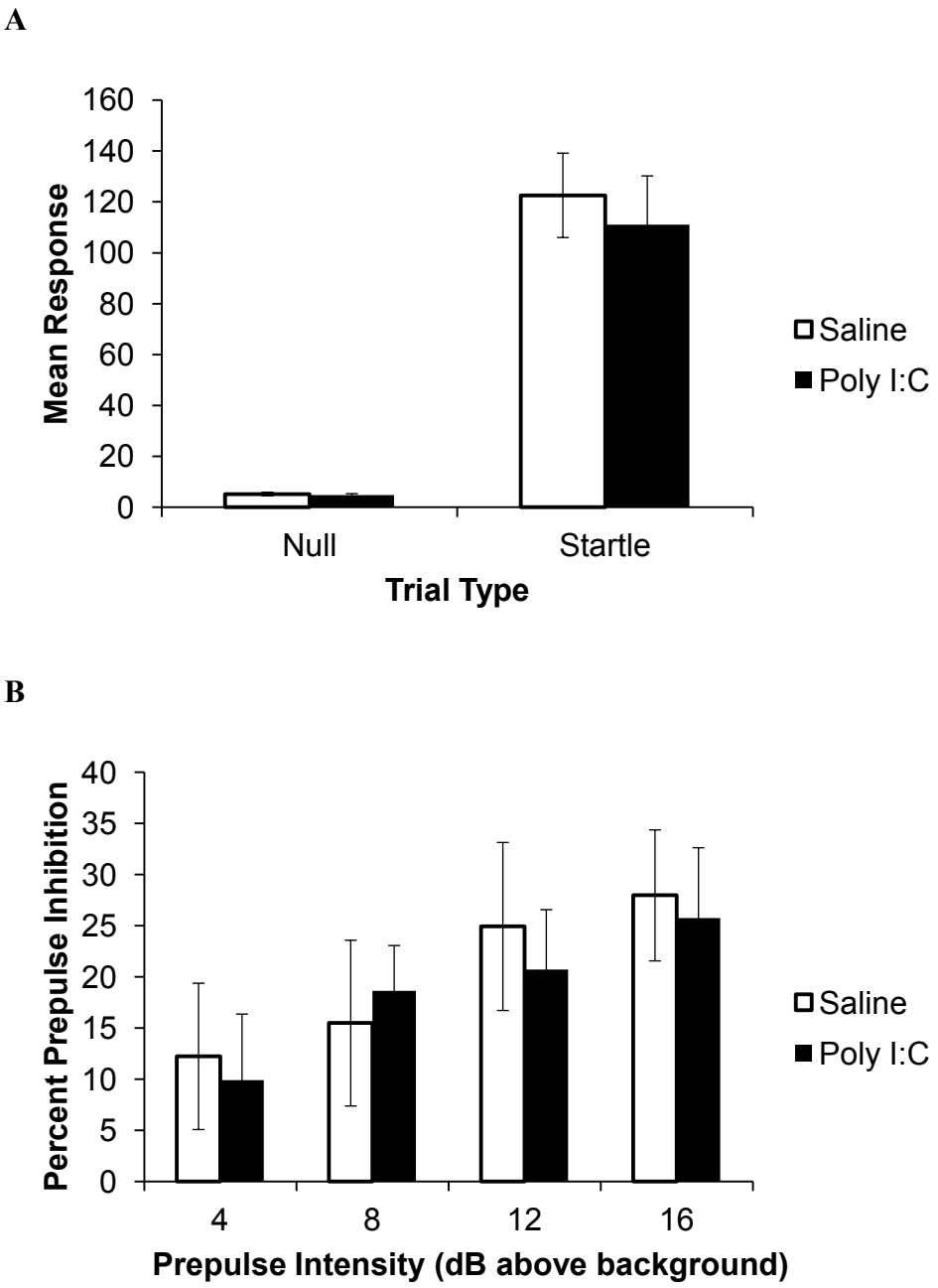


Figure 10. Mean (±SEM) of (A) magnitude of response in null trials and startle-only trials and (B) percent prepulse inhibition of the acoustic startle response in Saline and poly I:C female juvenile rats. *Significantly different, $p < 0.05$.

Hypotheses regarding NOP in male rats were not supported. During the juvenile period, male poly I:C rats showed NOP following a 90 sec delay while the SAL rats did not. Both the SAL and poly I:C male rats failed to show NOP following 15 min and 4 h delays. However, in adulthood, both the SAL and poly I:C male rats showed NOP at the 90 sec and 15 min delays, suggesting that the rats recognized the familiar object after these delays. Following the 4 h delay however, neither the SAL nor poly I: C males showed NOP possibly suggesting that they could no longer recognize the familiar object after such a long delay. Contrary to our hypothesis, NOP deficits were not limited to rats born to poly I:C-treated dams. The same pattern of results was observed in juvenile female rats where no object preference was observed in either of the groups following all delays. As was the case with adult males, both SAL and poly I:C adult females showed NOP following 90s and 15 min delays. Interestingly, our hypothesis was supported following a 4 h delay where the SAL females showed NOP while the poly I:C females did not, suggesting that prenatal exposure to poly I:C disrupted object recognition in females at a longer delay.

Our overall hypotheses concerning AMPH-induced locomotor activity were not supported. As expected, there were no differences between SAL and poly I:C juvenile male rats following saline injections. Following both the 0.75 mg/kg and 1.0 mg/kg AMPH challenges, there were no differences between the two treatment groups, though there appeared to be a trend for higher locomotor activity in the poly I:C rats compared to SAL rats. This trend disappeared, however, in adult male rats where no differences between SAL and poly I:C rats were observed following any of the challenges. In juvenile female rats, as hypothesized, there were no differences between treatment groups

following any of the injections. Interestingly and contrary to our hypothesis, the poly I:C adult female rats exhibited lower locomotor activity compared to the SAL adult females following the 0.75 mg/kg AMPH challenge, suggesting that prenatal exposure to poly I:C decreased sensitivity to the locomotor activating effects of AMPH. However, this result was not observed following the 1.0 mg/kg AMPH challenge.

Our hypotheses regarding PPI of the acoustic startle response in juvenile rats were supported. In both juvenile male and female rats, louder prepulses resulted in higher percent PPI, suggesting that louder prepulses were more effective at inhibiting the startle response to the pulse. As expected, there were no differences between SAL and poly I:C rats in percent PPI of the acoustic startle response suggesting that sensorimotor gating was not impaired. Unfortunately, these results are inconclusive as we were unable to assess for deficits in PPI of the acoustic startle response in adult rats.

CHAPTER 2

BEHAVIOURAL AND NEUROCHEMICAL CONSEQUENCES OF CHRONIC PRENATAL MK-801 ADMINISTRATION IN THE JUVENILE AND ADULT RAT OFFSPRING

Stephanie Gallant, Loïc Welch, Patricia Martone, Ashraf Mahmud, Uri Shalev

Introduction

As previously discussed, the use of the NMDA receptor antagonist, PCP, as an anesthetic in the 1950s revealed that NMDA receptor blockade resulted in hyperactivity, hallucinations, and cognitive impairment in healthy individuals (Bakker & Amini, 1961; Davies & Beech, 1960; Luby et al., 1959). Subsequent research illustrated that PCP exacerbated these existing symptoms in patients with schizophrenia (Neill et al., 2010). What followed was the birth of the glutamate hypothesis of schizophrenia which posits that symptoms of schizophrenia are, in part, the result of a hypoglutamatergic state that is mediated by NMDA receptor hypofunction (Duncan et al., 1999).

This hypothesis does not exclude the DA hypothesis but rather proposes a way in which both neurotransmitter systems may interact to produce the symptoms observed in schizophrenia. In fact, the observation that both typical and atypical antipsychotic drugs, which act primarily as DA antagonists, are effective in alleviating symptoms induced by NMDA receptor antagonism (e.g. social withdrawal, anhedonia, avolition) suggests an interaction between the two systems (Duncan et al., 1999). In addition, NMDA receptor blockade has been shown to result in increased striatal DA release which, in animals, results in increased locomotor activity (Tang et al., 2006) and cognitive impairment (Jentsch, Tran, Taylor, & Roth, 1998). Thus, the current hypothesis is that glutamatergic hypoactivity, via NMDA receptor hypofunction, may have downstream effects on the dopaminergic system resulting in a hyperactive mesolimbic DA system (Adams & Moghaddam, 1998). To better comprehend the putative role of the NMDA receptor in schizophrenia, one must have a clear understanding of this interesting receptor's structure and functioning.

The NMDA receptor (Figure 11) contains two subunits: NR1, which is required for channel function, and NR2A-D, which is involved in the pharmacologic characteristics of the NMDA receptor (Monaghan & Jane, 2009). In addition to the glutamate recognition site, a second site on the NR1 binds glycine and/or D-serine and must be occupied for glutamate to open the channel (Monaghan & Jane, 2009; Neill et al., 2014). At resting membrane potential, the NMDA receptor is blocked my $Mg^{2+,}$ which can be removed once the cell is depolarized. Within the channel is a binding site for dissociative anaesthetics such as PCP, ketamine, and MK-801, which act as non-competitive antagonists (Monaghan & Jane, 2009; Neill et al., 2014).

In order to experimentally examine the role of NMDA receptors in the pathophysiology of schizophrenia, researchers have assessed schizophrenia-like behaviours in response to treatment with receptor antagonists in rodents. The general procedure involves injecting the adult rat or mice with an NMDA receptor antagonist and assessing, often immediately after the drug treatment, behaviours that are thought to model negative, cognitive, and to a limited extent, positive symptoms of schizophrenia.

Research shows that prolonged exposure to PCP in adult mice results in increased immobility in the forced-swim test (Noda, Yamada, Furukawa, & Nabeshima, 1995). Although there is some controversy about whether increased immobility demonstrates adaptive learning rather than a depression-like symptom (Borsini, Volterra, & Meli, 1986), the forced-swim test is a common procedure used in research with rodents to assess depression-like behaviour (i.e. negative symptoms) (Xu et al., 2005). In this task, rats or mice are placed into a water-filled cylinder and expected to swim around the

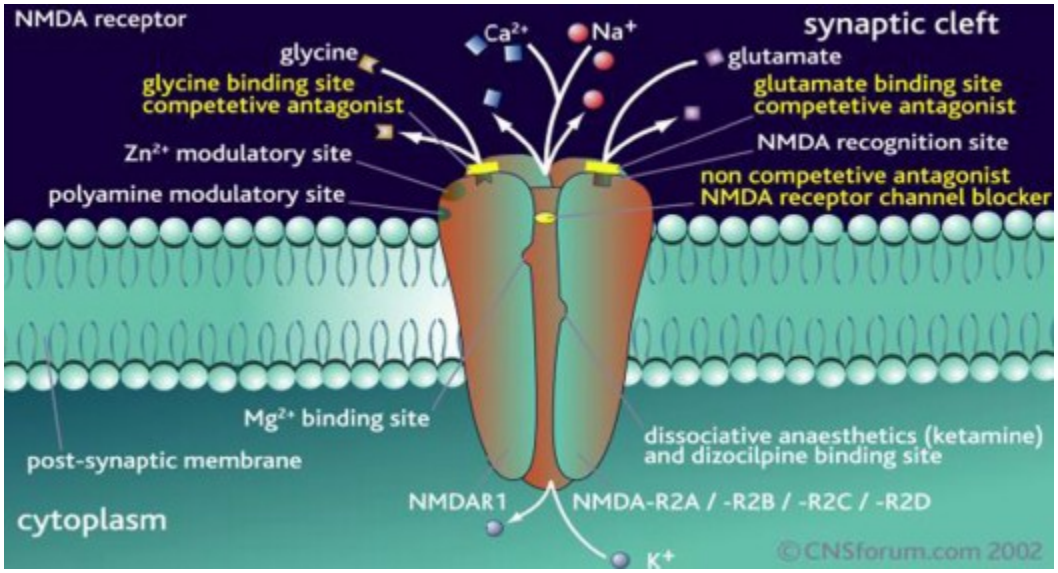


Figure 11. N-methyl-D-aspartate (NMDA) receptor. Figure taken from Neill et al. (2013).

cylinder walls as a survival instinct. Decreased latencies to immobility or increased immobility are thought to indicate depression-like behaviours (Borsini et al., 1986).

As previously discussed, perseveration, the difficulty in inhibiting behaviours on a learned task, is a cognitive symptom associated with cognitive flexibility and frequently reported in patients with schizophrenia. Researchers have shown that acute injections of MK-801 result in perseverative behaviour in rats trained in an operant chamber (Holahan, Clarke, & Hines, 2010). In addition, researchers have found that NMDA receptor antagonism via MK-801 in rats aged PND 7 through 10 induced set-shifting deficits in rodents assessed in a maze-based set-shifting task (Stefani & Moghaddam, 2005). In addition to perseveration, working memory is also reported to be impaired in schizophrenia and can be disrupted in rodents exposed to an NMDA receptor antagonist (Stefani & Moghaddam, 2005). PCP-treated rats have been shown to display impaired performance in a working memory task using a T-maze (Adams & Moghaddam, 1998 (Stefani & Moghaddam, 2005). Finally, both PCP and MK-801 have been shown to disrupt object recognition and spatial memory in mice and rats (Neill et al., 2010).

Although these models of schizophrenia-like behaviours using acute challenges with NMDA receptor antagonists have been useful in elucidating the role of glutamate, they fail to capture the neurodevelopmental nature of schizophrenia. Given the evidence pointing to early developmental aberrations resulting in symptoms observed in adulthood, it is tempting to assume that prenatal manipulation of the glutamate system would result in structural and functional changes in the adult offspring brain. Indeed, researchers have begun to expand the acute NMDA receptor antagonist models of schizophrenia-like

behaviours to include prenatal challenges to this system in an attempt to evaluate how this system may be linked to the neurodevelopmental etiology of schizophrenia.

The results, thus far, suggest that prenatal disruption of the glutamatergic system via NMDA receptor blockade may indeed be linked to schizophrenia-like behaviours in adult offspring. For instance, rats born to MK-801-treated dams (GD 15 – 18) show reduced post-pubertal density of parvalbummin-immunoreactive GABA neurons in the medial prefrontal cortex (Abekawa, Ito, Nakagawa, & Koyama, 2007). In addition, these adult offspring showed increased sensitivity to the locomotor activating effects of PCP (Abekawa et al., 2007). Another study found that prenatal PCP treatment (5 mg/kg/day; GD 6 – 18) in mice resulted in impaired object recognition memory and increased immobility in the forced-swim task in adult offspring (Lu et al., 2011). These behavioural changes were accompanied by decreased extracellular glutamate in the PFC, and increased expression of a glial glutamate transporter (GLAST) in the PFC. Furthermore, when a GLAST antagonist was administered, prefrontal glutamatergic neurotransmission was enhanced resulting in reversal of the observed behavioural deficits were reversed (Lu et al., 2011). In an earlier study, Lu et al. (2010) showed that prenatal exposure to PCP in mice resulted in increased expression but reduced phosphorylation of NR1. These results suggest that prenatal exposure to PCP result in schizophrenia-like symptoms and abnormal expression and dysfunction of NMDA receptors in adult mice (Lu et al., 2010). In addition to these studies, unpublished findings from our collaborators suggest that chronic prenatal exposure to MK-801 (0.1 mg/kg/day; GD 7 – 19) result in absence of novel object preference and altered locomotor response to an acute injection of MK-801 and amphetamine in the adult male offspring. Furthermore,

pilot studies from our laboratory suggest that prenatal exposure to MK-801 results in suppressed latent inhibition and shorter latencies to immobility in the forced swim test in the adult offspring.

Although the evidence thus far appears to suggest that prenatal alterations in the development of the glutamatergic system may be relevant to the etiology of schizophrenia, the adverse effects of prenatal exposure to NMDA receptor antagonists on a wide range of behaviours are not well understood. Furthermore, the effects of such a prenatal manipulation on behaviour have been assessed in adult, but not juvenile rats. Therefore, the aim of Chapter 2 was to 1) replicate the effects of prenatal MK-801 administration on locomotor activity and object recognition memory in adult rats; 2) expand on these findings by exposing the adult offspring to a large battery of behavioural tasks designed to model positive, negative, and cognitive-like symptoms; and 3) assess the effects of prenatal MK-801 exposure on behaviour in juvenile offspring. We hypothesized that rats born to MK-801-treated dams would show schizophrenia-like symptoms compared to control rats, in adulthood, but not in the juvenile period.

Materials & Methods

Subjects

Female Long-Evans rats (n = 15, 225-250 g) were purchased from Charles River (St. Constant, Quebec, Canada). Rats were housed three per cage under a 12 h light-dark cycle (lights on at 8:00 AM; 21˚C) with *ad libitum* access to food and water and were allotted 1 week of acclimation prior to breeding. One male breeder Long-Evans rat (n = 5, 350-400 g) was added to each cage. Vaginal smears were taken daily to monitor the female's estrous cycle and identify the period at which they were receptive. Females

were weighed daily and examined for vaginal plugs (indicating mating). The presence of a vaginal plug was defined as gestation day (GD) 0. At GD 6, females were housed individually in clear standard shoebox cages for the remainder of gestation. From GD 7 to 19, dams received daily subcutaneous (s.c.) injections of either 0.9% saline (n = 6) or 0.1 mg/kg MK-801 (n = 9). One day following parturition, litters were culled down to 12 pups per cage (6 males and 6 females when possible) to control for litter size effects. At PND 21, pups were weaned and housed three per cage with their respective treatment group. Different sets of rats were tested during the juvenile period (PND 30-40) and adulthood (PND 70+). All behavioural testing was conducted during the light phase of the cycle. All experiments were performed in accordance with the guidelines of the Canadian Council of Animal Care and all animal procedures were approved by the Animal Research Ethics Committee of Concordia University.

Apparatus & Procedure

Maternal behaviour and pup weights. Maternal behaviour of saline (SAL; n = 5) and MK-801 (MK) treated dams (n = 5) was observed twice a day each day for the first 14 days following parturition to assess whether the prenatal manipulation resulted in differences in dam behaviour. As described in Chapter 1, a rater made observations once per minute in 10-min increments at two different times (09:30 and 16:00 hrs). Maternal behaviours observed included arched-back nursing, nursing prone, grooming/licking, nest building, and off-nest. Maternal behaviours were converted to a percentage of the number of times the dam engaged in each of these behaviors over the total number of time points observed. Pups were weighed at PND 2, 7, 14, and 21.

Novel object preference. The same apparatus and procedure described in Chapter 1 to assess NOP was used in the present experiment. The task was divided into the three phases of habituation, familiarization, and retention test. The delays used between the familiarization phase and retention test with juvenile rats (Males: SAL, *n* = 9; MK, *n* = 11; Females: SAL, *n* = 6; MK, *n* = 8) were 90 sec, 15 min, and 4 hrs. Delays used with adult rats (SAL, *n* = 9; MK, *n* = 11; Females: SAL, *n* = 8; MK, *n* = 11) were 15 min, 90 min, and 4 hrs. Investigation times were recorded using ODLog version 2.7.2 (Macropad, software) and an investigation ratio ($t_{novel}/(t_{novel} + t_{familiar})$) was computed for the retention test.

Drug-induced locomotor activity. Locomotor activity was quantified with the same infrared activity-monitoring apparatus described in Chapter 1. Data were captured using Trusacan2 software in 5-min time intervals for total distance traveled (successive beam interruptions (cm)). All rats were tested throughout the experiment in the same respective activity chamber at the same time of day.

Juvenile. SAL (Males: *n* = 7; Females: *n* = 7) and MK (Males: *n* = 9; Females: *n* = 8) rats were tested during the juvenile period. On day 1, rats were placed in the box for 30 min prior to injection. Following this habituation period, all rats received an injection of 0.9% saline (i.p.) and were left in the activity chamber for an additional 90 min. On days 2 and 3, rats underwent the same procedure but received an i.p. injection of 0.1 mg/kg and 0.2 mg/kg MK-801 respectively. After a 4 day washout period, rats were again tested under the same procedure but received an injection of saline (day 1), 0.75 mg/kg amphetamine (day 2), and 1.0 mg/kg amphetamine (day 3).

Adults. Drug-naive SAL (Males: *n* = 8; Females: *n* = 8) and MK (Males: *n* = 8; Females: *n* = 8) rats were tested during adulthood. Rats were tested under the same protocol used in the juvenile period with a few dose adjustments. All rats first received i.p. injections of saline on day 1 and 0.1 mg/kg MK-801 on day 2. On day 3, the females received an i.p. injection of 0.2 mg/kg MK-801 while the males received 0.4 mg/kg MK-801. Following a one week washout period, all rats were tested under the same protocol but this time received saline (day 1), 0.75 mg/kg amphetamine (day 2), and 1.0 mg/kg amphetamine (day 3). Males underwent an additional day of testing with a 1.5 mg/kg amphetamine challenge (day 4).

Delayed non-match to place task. Working memory was assessed using an opaque T-maze placed on a table one meter above the ground. The T-maze walls extended 28 cm above a wire grid flooring which measured 10.5 cm in width. The roof of the maze was transparent. Three arms were arranged at 90° angles around a 14 x14 cm central chamber and measured 75 cm in length. Black polyurethane guillotine doors were used to open or close the entrance to the maze and to the goal arms from the central chamber. Using a string attached on the ceiling above the maze, these gates could be lifted from a remote location thereby ensuring that the experimenter remained in the same location throughout the experiment. The experimenter always stood directly behind the start box during training and testing. A metal bowl was placed at the end of each goal arm and a food reward (1/4 of a Kellogg's ®Froot Loops) was placed inside when appropriate. Additionally, Froot Loop crumbs were placed directly underneath the maze in an effort to avoid confounds associated with olfactory cues. The training and testing procedures took place in a dim-lighted room.

Adult male rats (n_{SAL} = 7, n_{MK} = 8) were placed on a food restriction diet in order to increase the salience of the reward. Once rats reached 90% of their initial body weight, DNMTP began and consisted of three phases: habituation, acquisition, and testing.

Habituation. For three consecutive days, rats were allowed 15 min of exploratory behavior within the maze.

Acquisition. Following the habituation sessions, acquisition training began. Ten sessions were carried out daily. Within each session, there were two phases: the forced-trial and the choice-trial. During the forced-trial, one arm of the maze was closed, the other left opened and baited with a quarter of a Froot Loop. The rat was placed in the start arm and allowed to enter the open arm and consume the reward. Immediately after consuming the reward, the rat was removed from the maze and placed in a yellow bucket with a white cover that was placed on the ground next to the maze for approximately 5 sec. During the choice-trial, the opposite arm was baited and both arms were open. Rats had to remember the arm visited during the forced-trial in order to correctly alternate during the choice-trial to obtain the food reward. The baited arm was randomly assigned to either the right or left arm for no more than three successive right or left designations per session. Once the rat performed 9/10 correct trials for three consecutive days, they were considered to have met criteria for testing.

Testing. The day after criterion had been met, rats underwent the testing which followed the training protocol described above, except three delays (5, 15, and 20 min) were introduced between the forced and choice phases of each of the ten daily trials. Twenty-one days following the last test, rats were again tested with a 20 min delay. The

following day, rats received an i.p. injection of 0.75 mg/kg amphetamine 20 min prior to the forced phase and were tested in the choice phase after a 5 min delay.

Social interaction. The social interaction test was performed in a square open-field box made of wood (123 x 123 x 46 cm) placed directly on the ground. The floor was covered with wood chip bedding and a web camera was positioned above the arena in order to record the sessions for later analysis.

Adult male rats were paired with an unfamiliar rat from the same treatment condition (n_{MK} = 8; n_{SAL} = 8) matched for body weight (within 15 g). All rats were habituated to the test environment and arena prior to the test day. Habituation consisted of placing each rat in the arena for 10 min on two consecutive days. On test day, pairs of rats were placed in the test arena together for 10 min. Behaviour during test day was recorded for subsequent scoring with ODLog version 2.7.2 (Macropad, software). The following four parameters were scored: (1) following (rat moves after the conspecific around the arena); (2) sniffing (sniffing the conspecific's snout or parts of the body including the anogenital region); (3) mounting (climbing over the conspecific's back); and (4) wrestling (fighting with the conspecific).

Sucrose preference. Anhedonia-like behaviour was assessed in adult male rats (n_{MK} = 10; n_{SAL} = 10) using the sucrose preference test under water-restricted conditions and under non-restricted conditions. Under both conditions, rats were tested with a 1% and a 5% sucrose solution, the order of which was counterbalanced.

Water restricted condition. Rats were water restricted and given access to two identical bottles of tap water from 9:00 to 9:15 and 17:00 to 17:45 for 5 days. Water consumption was recorded every morning following the 15 min water access by

reweighing preweighed bottles to ensure that the rats were drinking during this period and were not showing a preference for the right or left bottle. All sucrose preference tests took place at 9:00 and the bottles were reweighed at 9:15. The side on which the bottle of sucrose solution was placed was counterbalanced. The first test took place the day after the 5 days of water restriction. Half the rats were given access to a bottle of tap water and a bottle of 1% sucrose solution while the other half were given a bottle of tap water and a bottle of 5% sucrose solution. The following day, rats that had previously been given access to 1% sucrose were now given 5% sucrose and vice versa.

Non-Restricted Condition. Rats were given free access to two bottles of water for the remainder of the experiment. Under non-water restricted conditions, rats were again presented with a bottle of either 1% or 5% sucrose solution (counterbalanced) and a bottle of tap water for a period of 4 hours (9:00 to 13:00). The preweighed bottles were reweighed after 15 min and 4 hrs. The percent sucrose preference was calculated using the following formula: Sucrose preference = sucrose consumption/(water consumption + sucrose consumption) x 100%.

Set-shifting. Cognitive flexibility was assessed in adult male rats (n_{MK} = 8; n_{SAL} = 7) using a plus-maze based set-shifting procedure. The plus-maze walls extended 28 cm above a wire grid flooring which measured 10.5 cm in width. The roof of the maze was transparent. Four arms were arranged at 90° angles around a 14 x14 cm central chamber and measured 75 cm in length. Black polyurethane guillotine doors were used to open or close the entrance to the maze and to the goal arms from the central chamber. At the end of each arm was a metal food recipient. One of the arms was paneled with

white plastic serving as a visual cue. The task was divided into four phases: habituation, turn bias procedure, pre-shift training, and post-shift training.

Habituation. On day 1**,** three Froot Loops were placed in each arm with two pieces in the food well at the end of the arm. Rats were allowed to move freely in the maze for 15 min**.** If all 12 Froot Loops were eaten in less than 15 min, it was placed in a yellow holding bucket while the arms were re-baited before it was placed back into the maze for the remaining time. On day 2, the same procedure as day 1 was repeated, however, when the rat ate two Froot Loops, it was removed from the maze and placed into another arm. This was done in an effort to acclimatize the rats to being handled after eating the cereal reward. The subsequent habituatation sessions were the same as on day 2 except only two half loops were placed in the food wells only. This procedure continued daily until the rat consumed the rewards from all food wells for four trials. One trial consisted of the rat consuming all the rewards in every arm.

Turn-bias. Once habituation was complete, rats were tested to establish their turn bias. The maze was turned into a t-maze by blocking off one of the arms. The rat was placed at the beginning of the start arm and allowed to turn left or right to obtain a half Froot Loop from the food well at the end of the arm (both arms were baited). Once the rat turned and consumed the reward, it was picked up, placed in the same start arm, and allowed to make another choice of arm. If the rat chose the same initial arm, it was returned to the starting arm until it chose the opposite arm from its initial choice and ate the cereal. After choosing both arms, the rat was returned to the yellow holding bucket, the block and visual cue were moved to different arms and a new trial began. The turn bias trial consisted of entering both choice arms and consuming the cereal for a total of 7

trials. Each initial turn was recorded and summed up at the end of the 7 trials to provide a turn-bias (4 or more turns in the same direction out of 7). This turn bias was later used for the response-discrimination testing where the rat was trained to turn the opposite direction of its turn bias.

Response-Visual-Cue Testing. The task was separated into two phases: pre-shift and post shift. In each phase, rats had to learn a different rule which was counterbalanced. In both phases, the rat started from one of the arms designated west, south, or east. One of the arms had white paneling which served as a visual cue and was placed in different arms pseudo-randomly. For every consecutive set of 12 trials, the visual cue appeared an equal number of times in each arm. During the pre-shift phase, the rats had to either learn response or visual-cue discrimination (counterbalanced). For response discrimination, the rat was placed in a starting arm (never north) and had to learn to turn in the opposite direction of its turn-bias in order to reach the food well with a half-piece of Froot Loops. For visual-cue discrimination, the rat had to learn to turn into the arm with the visual cue to obtain the reward (acquisition). This procedure was repeated for 24 trials daily until the rats successfully achieved 10 consecutive trials. Once this was achieved, the rat was tested in a probe phase, where the rat started from the north arm, which had never used before to avoid possible visual cues in the room. If the rat correctly turned in the same direction as on testing, the pre-shift phase was completed. However, if the rat made an incorrect turn, training continued as described above until the rat made 5 consecutive correct choices at which point another probe trial was administered. This procedure continued until the probe was successfully completed. Once the pre-shift phase was completed, the post-shift phase began. In the post-shift

phase, rats had to ignore the previously relevant rule in order to acquire a new rule. Rats that had previously been trained in response discrimination were now trained on visual cue discrimination and vice versa. The same procedure as described above was implemented here where the rat had to achieve 10 correct trials consecutively and a final probe. The procedure is based on Ragozzino (2001) (Figure 12).

High performance liquid chromatography. Concentrations of DA, 3,4-dihydroxyphnylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by electrochemical detection.

Tissue preparation. Adult male rats (n_{MK} = 8; n_{SAL} = 7) received an i.p. injection of 0.75 mg/kg amphetamine. Twenty min after injection, rats were decapitated without anesthesia and their brains were removed and immersed in 2-methylbutane, chilled with dry ice, for 15 s and stored at -80˚C. Bilateral tissue punches of 1 mm diameter were excised from 1.5 mm thick coronal slices (Figure 13). Six punches from each hemisphere were taken for the nucleus accumbens (NAcc), dorsal lateral striatum (dlSTR), and dorsal medial striatum (dmSTR). Three punches from each hemisphere were taken for the ventral prefrontal cortex (vPFC). Filtered 0.1 M phosphate buffer was added to the tissue (100 µl) and the sample was vortexed for 30 s and re-frozen at -80 °C.

One day before conducting high-performance liquid chromatography (HPLC), tissue samples were left to thaw for 1 hr on ice, vortexed for 30 s, and then centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was separated from the protein pellets and filtered (0.45µm). Both the supernatant and the protein pellets were re-frozen at -80 °C.

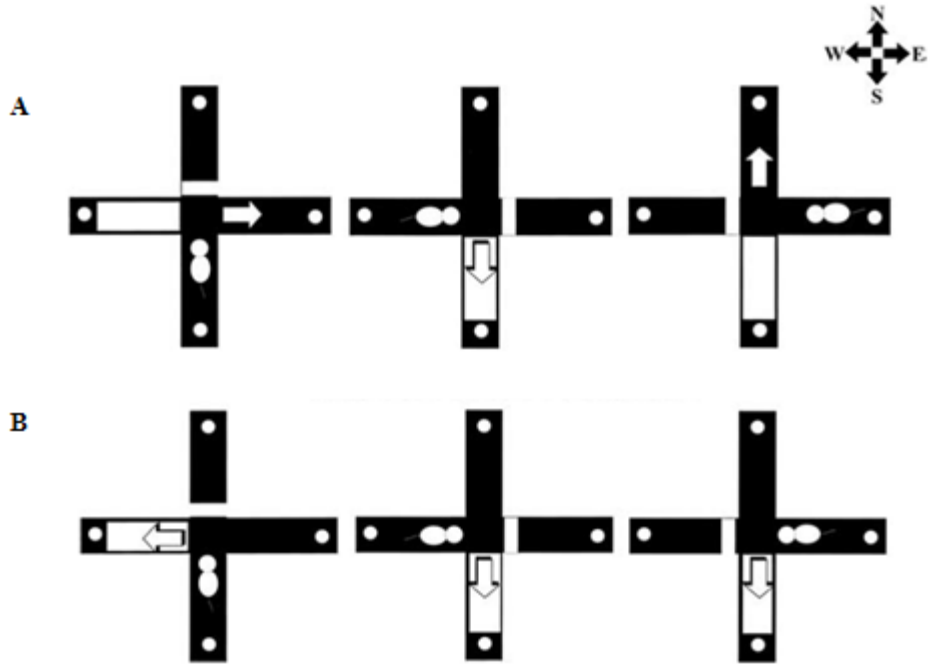
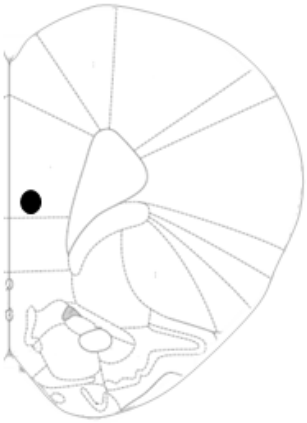
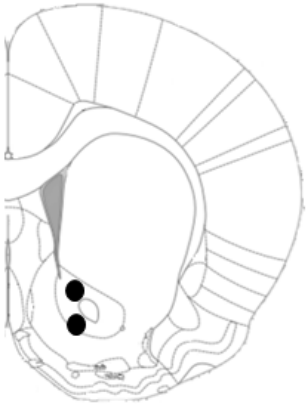


Figure 12. Maze-based set-shifting task. A) Response discrimination, B) Visual-cue discrimination. Image adapted from (Ragozzino & Kesner, 2001)



+ 3.24 mm (vPFC)



+ 1.56 mm (NAcc)

+ 1.20 mm (dmSTR & dlSTR)

Figure 13. Location of tissue of tissue punches. Bilateral tissue punches of 1 mm diameter were excised from 1.5 mm thick coronal slices.

HPLC. DA and its metabolites were separated from other chemical species in the dialysate samples using HPLC and quantified using electrochemical detection (ED) as described in (Hernandez, Rajabi, Stewart, Arvanitogiannis, & Shizgal, 2008). Dialysate samples were loaded through manual injection ports (Rheodyne 7125; Rheodyne LLC, Rhonert Park, CA; 20µl loop) into a reverse-phase column (15cm × 0.46cm Spherisorb-ODS, 5µm; Higgins Analytical, Mountain View, CA). Following separation in the column the sample passed through dual-channel ESA (Chelmsford, MA) coulometric detectors (Coulochem 5100, with a model 5011 analytical cell), which were connected to a computer. The detectors were set to reduce DA in one channel and to oxidize DA's metabolites, DOPAC and HVA, in the other channel. Standard samples of solutions containing known concentrations of DA and its metabolites were used to calibrate the equipment. Waters 515 HPLC pumps (Lachine, Quebec, Canada) were used to circulate the mobile phase (19% acetonitrile, 40mg 0.076M SDS, 0.1M EDTA, 0.058M $NaPO_4$, 0.03M citric acid, pH 3.35) at a flow rate of 1.2mL/min. EZChrom Chromatography Data System (Scientific Software Inc., San Ramon, CA) was used to analyze and integrate the data obtained for DA, DOPAC and HVA.

Protein quantification. Protein pellets were left to thaw for 1 hr on ice and 150 µl of NaOH was added to each sample, then vortexed for 30 s. Three microliters of the resulting solution were analyzed in duplicate from each sample using a microplate scanning spectrophotometer (FLUOstar Galaxy, BMG Labtechnologies) using the BCA Protein Assay Kit. Concentrations were estimated by comparing those from a known standard, then taking the average of the duplicate samples.

Drugs. For prenatal treatment: MK-801 (Sigma-Aldrich) was dissolved in 0.9% saline to create a solution of 0.1 mg/kg. For drug-induced locomotor activity: *D*-Amphetamine sulfate (AMPH) and MK-801 (both from Sigma-Aldrich) were dissolved in 0.9% saline.

Statistical Analysis

All statistical analyses were conducted using SPSS version 22 for Windows (IBM, Chicago, IL). As described in Chapter 1, corrected degrees of freedom where used when assumptions of equality of variance were violated and effect sizes were computed for all tests.

Novel Object Preference. A one sample *t*-test was used to compare each group's investigation ratio to chance (0.5). *Prenatal treatment* (SAL, MK) served as the independent variable while investigation ratio served as the dependent variable. Investigation ratios that were significantly above chance level indicated that rats displayed preference for the novel object, suggesting that rats recognized the familiar object.

Drug-Induced Locomotor Activity. Separate two-way mixed analyses of variance (ANOVA) were conducted for each dose, using *prenatal treatment* (SAL, MK) as the between-subject factor and *time* as the within-subject factor (18 x 5-min bins). The dependent variable was the total distance travelled (cm).

Delayed Non-Match to Place. A one sample *t*-test was used to compare each group's number of correct trials (out of 10 trials) to chance (5/10). *Prenatal treatment* (SAL vs. MK) served as the independent variable while correct trials served as the dependent variable. Number of correct trials that were significantly above chance

indicated that rats remembered which arm they had previously entered before the delay and were able to use their working memory adequately to enter the opposite arm during the choice phase.

Social Interaction. An independent samples *t*-test was used to compare prenatal treatment (SAL vs MK; independent variable) on the four following behaviours: following, sniffing, mounting, and wrestling.

Sucrose Preference. An independent samples *t*-test was used to compare *prenatal conditions* (SAL vs MK; independent variable) on total water consumption (tap water plus sucrose solution; dependent variable). A one sample *t*-test was used to compare each group's percentage of sucrose preference to chance (50%). *Prenatal treatment* (SAL vs. MK) served as the independent variable while percentage sucrose preference served as the dependent variable. Percentage of sucrose preference significantly above 50% suggested that rats preferred the sweetened water to the tap water while absence of sucrose preference was interpreted as indicating anhedonia.

Set-Shifting. Independent samples *t*-tests were conducted to compare the MK group to the SAL group on the following measures: (1) acquisition (the number of trials required for the rat to reach its first probe test); (2) criterion (number of trials until the rat successfully completed the probe); (3) probes (number of probes completed until correct response on probe test); and (4) null trials (number of trials during which the rat did not move for 5 consecutive min). These variables were analyzed for both the pre-shift and post-shift phases of the task. In addition, independent samples *t*-tests were conducted to

compare both treatment conditions on errors committed during the post-shift phase. Errors included: (1) never-reinforced errors (rat follows neither of the rules learned during response or visual cue discrimination); (2) regressive errors (rats are assumed to have learned the new rule, however sporadically revert back to the previous rule); and (3) perseverative errors (rats continue to use the previously learned rule when the trial requires turning the opposite direction). Perseverative errors were counted by dividing the trials into blocks of four trials and when the rat used the first acquired rule three times or more within one block, it was considered to be perseverative. When the rats used the first rule less than three times out of four within one block, the errors were counted as regressive.

High performance liquid chromatography. An independent samples *t*-test was used to compare prenatal treatment (SAL vs MK; independent variable) on DA turnover in the NAcc, dmSTR, dlSTR, and vPFC.

Results

Maternal behaviour and pup weights

There were no statistically significant differences between MK-801- and saline-treated dams on any of the five assessed maternal behaviours (Figure 14). Thus, the treatment condition did not affect the way in which dams cared for their offspring during the first 14 days of life. Body weights of MK and SAL male offspring did not differ significantly at PND 2, 7, 14, and 21 (Figure 15 A). However, MK female pups weighed significantly less compared to the SAL pups at PND 2, $t(41) = 2.93$, $p = .006$, $g = 0.91$, and 7, $t(39) = 2.91$, $p = .006$, $g = 0.91$. There were no statistically significant weight differences at PND 14 and 21 (Figure 15 B).

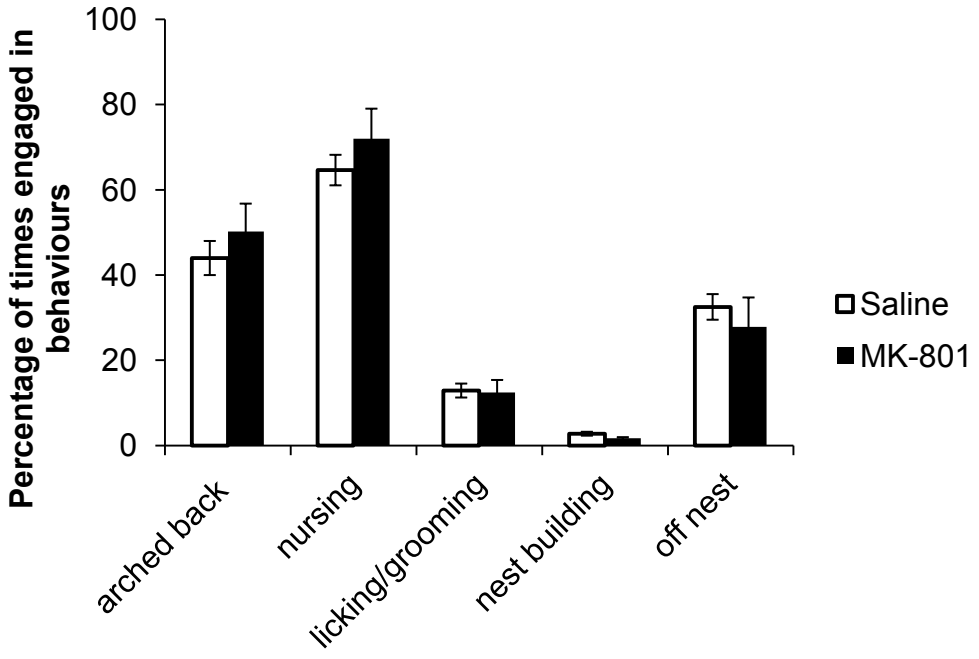


Figure 14. Mean (± SEM) percentage of number of times Saline and MK-801 dams engaged in maternal behaviours. From GD 7 to 19, dams received daily subcutaneous injections of either 0.9% saline or 0.1 mg/kg MK-801. Refer to text for detailed description of maternal behaviours.

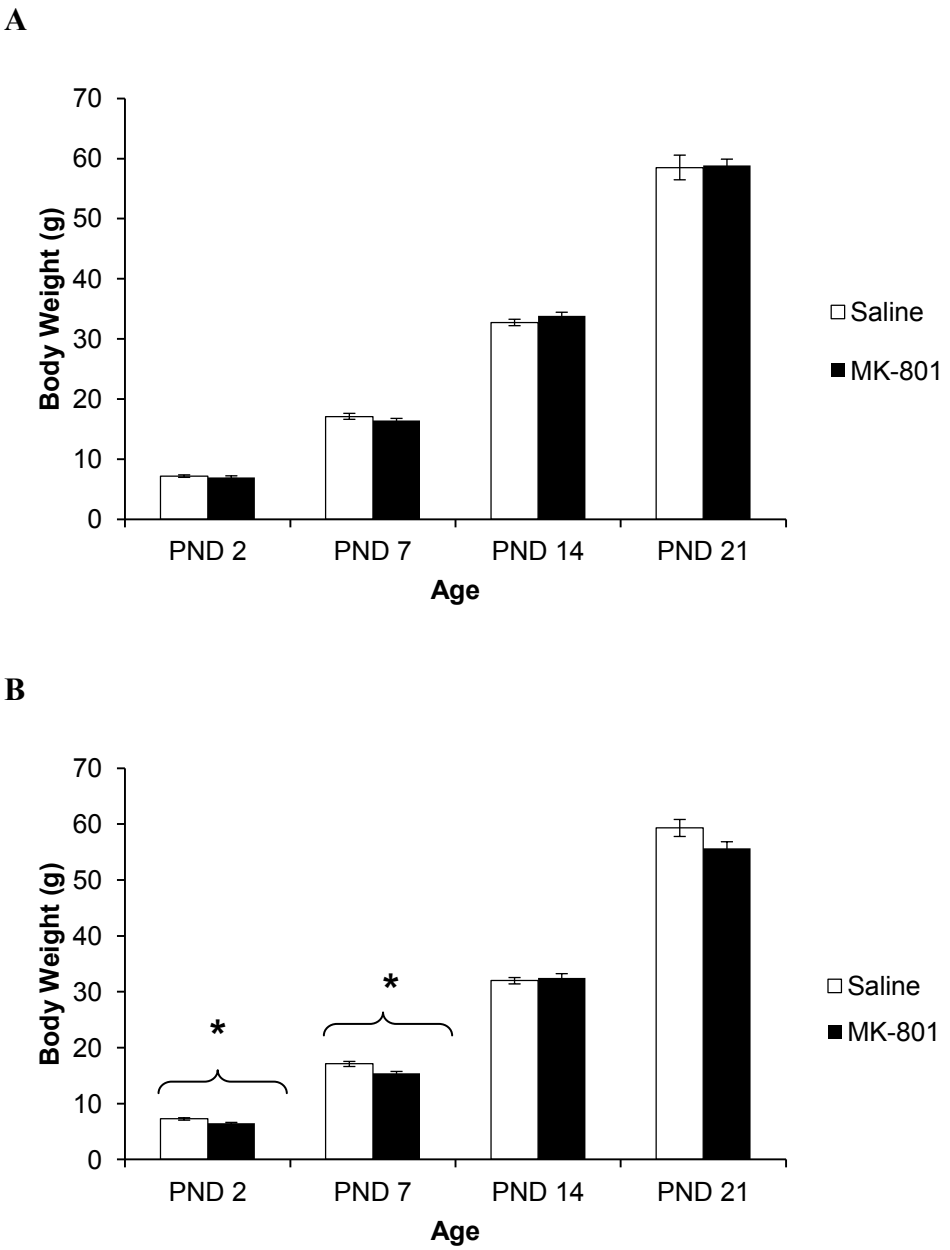


Figure 15. Mean (± SEM) body weights of (A) male and (B) female offspring from the Saline and MK-801 prenatal treatment groups at postnatal days 2, 7, 14, and 21. * $p <$ 0.05.

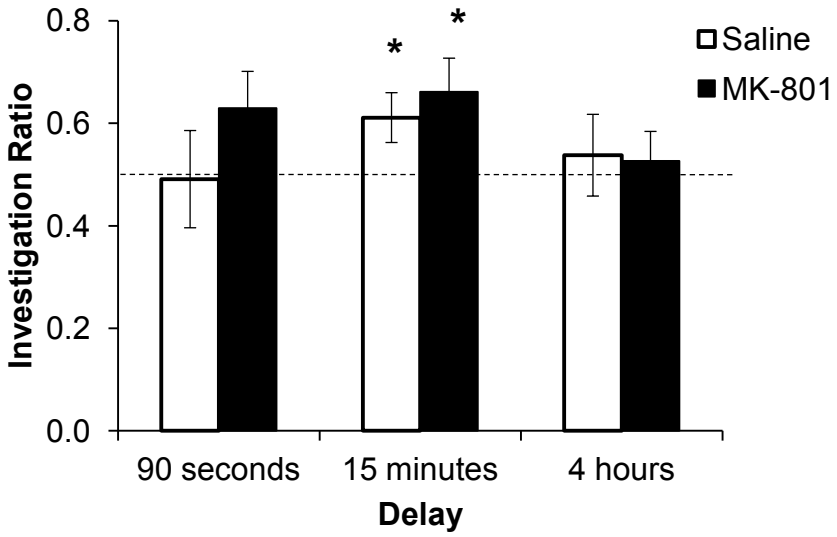
Novel object preference

Males

Juvenile. As seen in figure 16 A, following a 90 sec delay, neither the SAL, $t(8) = 0.10$, $p = .925$, $d = 0.03$, or the MK rats, $t(9) = 1.82$, $p = .103$, $d = 0.57$, showed a statistically significant preference for the novel object. However, following a 15 min delay, both the SAL, $t(7) = 2.28$, $p = .056$, $d = 0.81$, and the MK rats, $t(8) = 2.46$, $p = .039$, $d = 0.82$, showed a statistically significant preference for the novel object, suggesting that they recognized the familiar objects following this delay. After a 4 hr delay, neither the SAL, $t(8) = 0.47$, $p = .650$, $d = 0.16$, or the MK rats, $t(9) = 0.46$, $p = .657$, $d = 0.14$, showed a statistically significant preference for the novel object, suggesting that rats were unable to recognize the familiar object following the longer delay.

Adults. As seen in Figure 16 B, following a 15 min delay, both the SAL, $t(8) = 3.24$, $p = .012$, $d = 1.08$, and the MK rats, $t(10) = 2.90$, $p = .016$, $d = 0.57$, showed statistically significant preference for the novel object. After a 90 min delay, the SAL rats again showed statistically significant preference for the novel object, $t(8) = 2.90$, $p = .020$, $d = 0.97$. However, the MK rats did not show statistically significant preference at this delay, $t(10) = 1.56$, $p = .150$, $d = 0.47$, suggesting that they may not have recognized the familiar object. Following a 4 hr delay, neither the SAL, $t(8) = 0.33$, $p = .749$, $d = 0.11$, or the MK rats, $t(10) = 0.82$, $p = .431$, $d = 0.25$, showed statistically significant preference for the novel object.

A



B

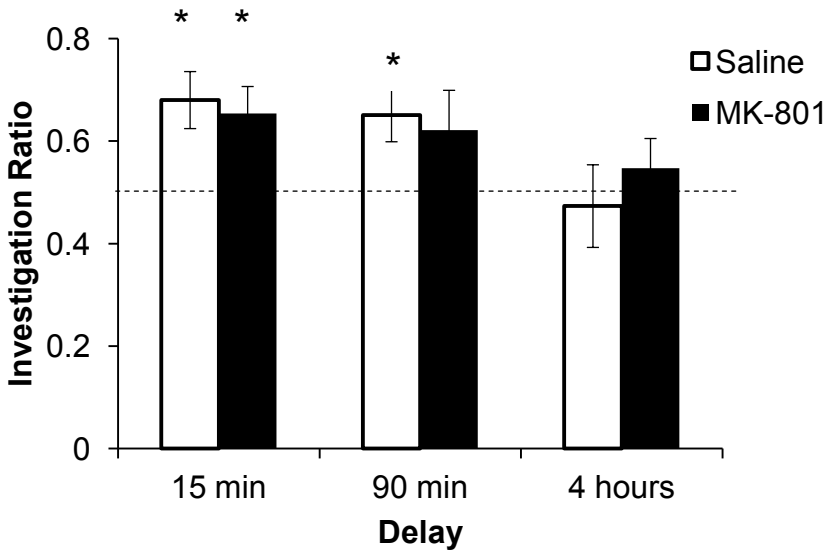


Figure 16. Mean (±SEM) investigation ratio for the first minute of the novel object preference test sessions for (A) juvenile prenatal Saline and MK-801 and (B) adult prenatal Saline and MK-801 male offspring. *Significantly different from 0.5, $p < 0.05$.

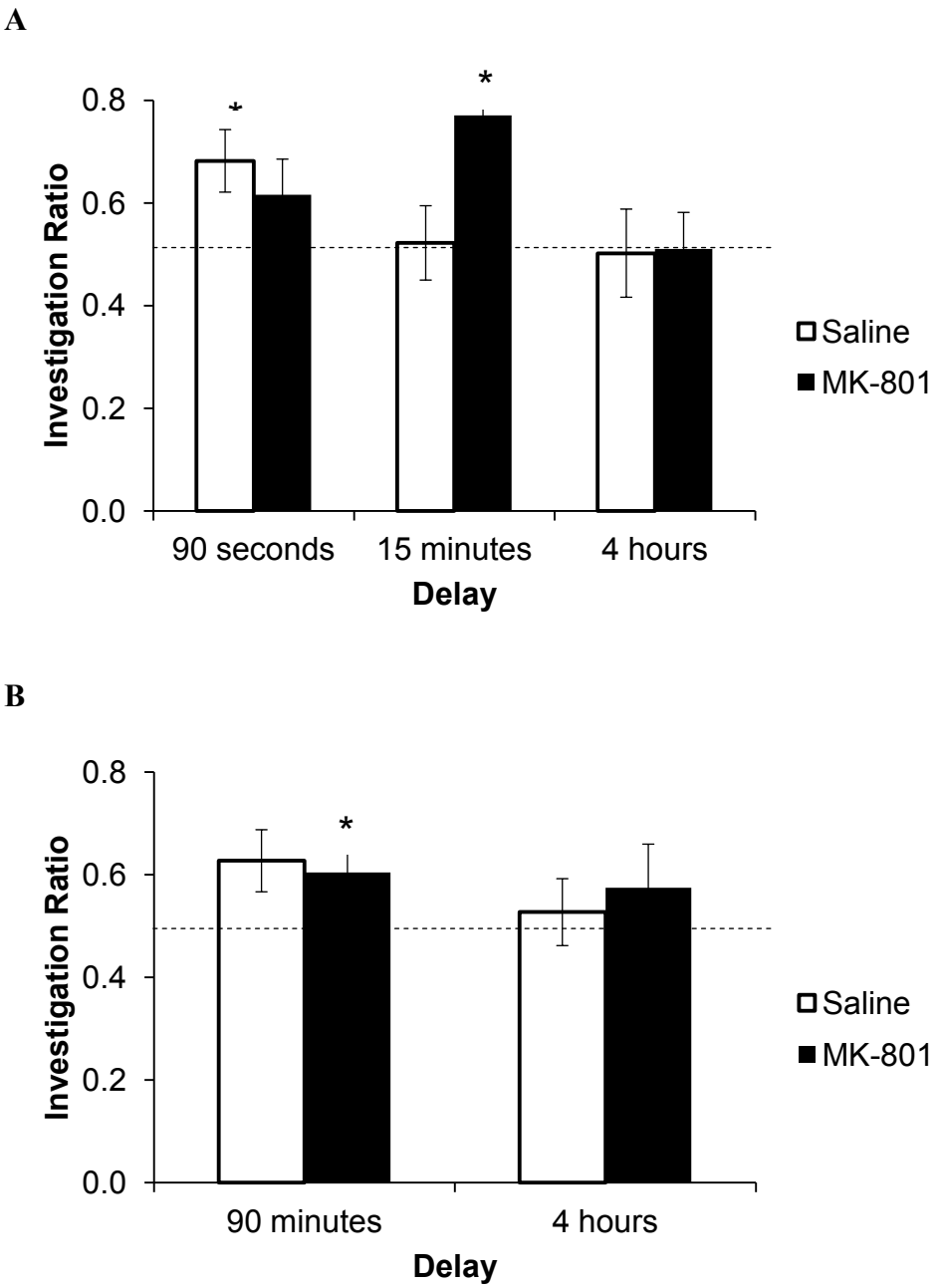


Figure 17. Mean (±SEM) investigation ratio for the first minute of the novel object preference test sessions for (A) juvenile prenatal Saline and MK-801 and (B) adult prenatal Saline and MK-801 female offspring. *Significantly different from 0.5, $p < 0.05$.

Females

Juvenile. As seen in figure 17 A, following a 90 sec delay, the SAL rats showed statistically significant preference for the novel object, $t(5) = 2.98$, $p = .031$, $d = 1.21$, while the MK rats did not, $t(7) = 1.69$, $p = .135$, $d = 0.60$. Conversely, after a 15 min delay, the SAL rats did not show statistically significant preference for the novel object, $t(3) = 0.30$, $p = .780$, $d = 0.15$, while the MK rats did, $t(7) = 9.74$, $p = .000$, $d = 3.44$. Following a 4 hr delay, neither the SAL, $t(5) = 0.02$, $p = .982$, $d = 0.01$, or the MK rats, $t(6) = 0.15$, $p = .886$, $d = 0.06$, showed statistically significant preference for the novel object.

Adults. As seen in Figure 17 B, following a 90 min delay, the SAL rats did not show statistically significant novel object preference, $t(10) = 2.10$, $p = .062$, $d = 0.63$, while the MK rats did, $t(7) = 2.84$, $p = .025$, $d = 1.00$. Following a 4 h delay, neither the SAL , $t(10) = 4.16$, $p = .686$, $d = 0.13$, or MK rats, $t(7) = 0.87$, $p = .413$, $d = 0.30$, showed statistically significant preference for the novel object.

Drug-induced locomotor activity

Juvenile males

MK-801-induced locomotor activity. Following the saline injection (day 1), ambulatory locomotion significantly habituated over time $F(17,238) = 15.19$, $p < .001$, $\eta_p^2 = 0.52$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.33$, $p = .573$, $\eta_p^2 = 0.02$ (Figure 18 A). The *time* by *prenatal treatment* interaction was statistically significant, $F(17, 238) = 4.07$, $p < .001$, $\eta_p^2 = 0.23$. The 0.1 mg/kg MK-801 challenge (day 2) induced a statistically significant increase in locomotor activity over time, $F(17, 238) = 8.24$, $p < .001$, $\eta_p^2 = 0.37$. However, there

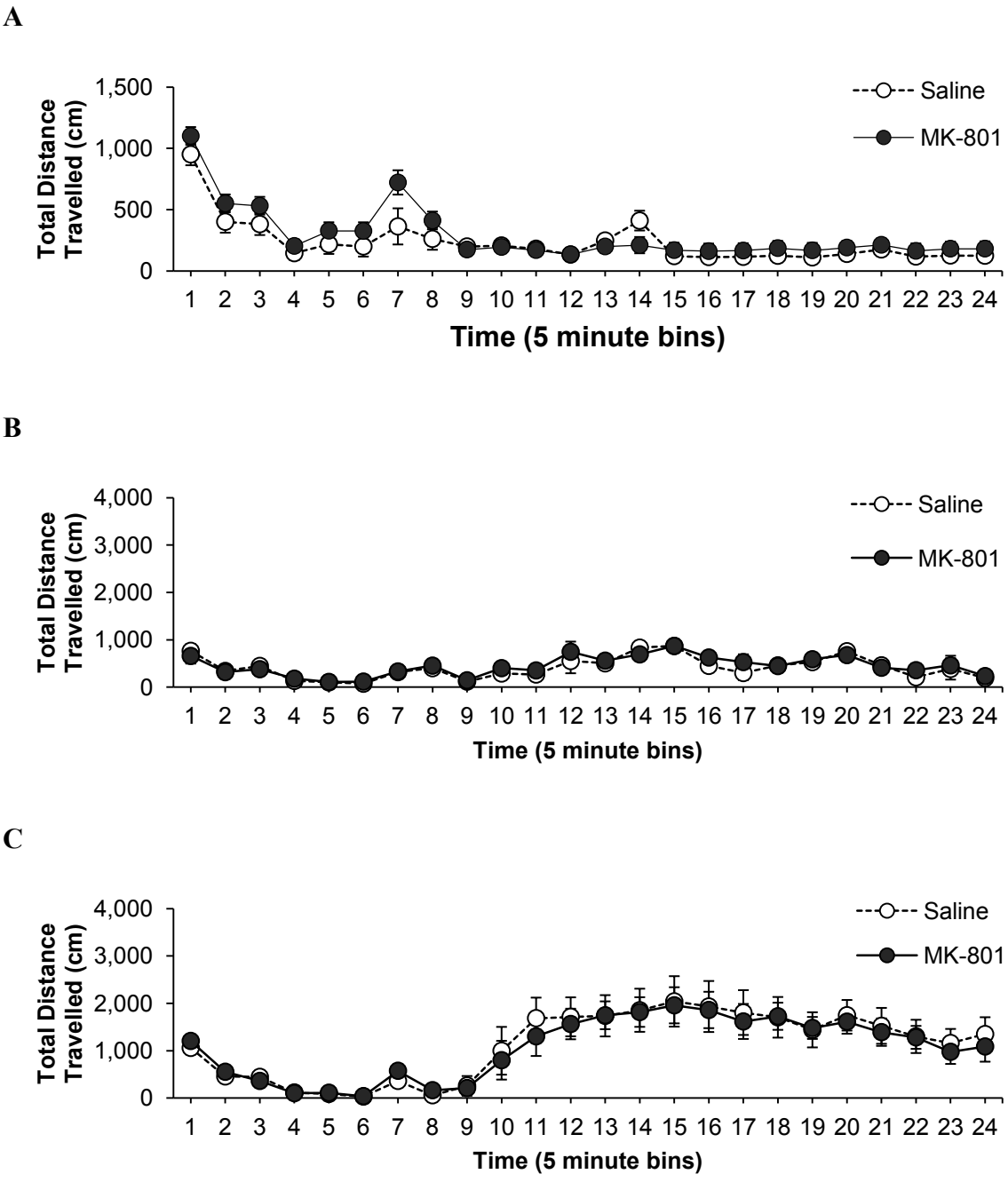


Figure 18. Mean (±SEM) total distance travelled (cm) by juvenile prenatal Saline and MK-801 male rats following a (A) saline injection, (B) 0.1 mg/kg MK-801 injection, and (C) 0.2 mg/kg MK-801 injection.

was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.27$, $p = .614$, $\eta_p^2 = 0.02$ and there was no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.50$, $p = .950$, $\eta_p^2 = 0.04$ (Figure 18 B). The 0.2 mg/kg MK-801 challenge (day 3) resulted in a statistically significant increase in locomotor activity over time, $F(17, 238) = 17.78$, $p < .001$, $\eta_p^2 = 0.56$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.04$, $p = .832$, $\eta_p^2 = 0.00$ and no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.27$, $p = .840$, $\eta_p^2 = 0.02$ (Figure 18 C).

Amphetamine-induced locomotor activity. The 0.75 mg/kg amphetamine challenge (day 1) resulted in a statistically significant increase in locomotor activity over time, $F(17, 238) = 23.7$, $p < .001$, $\eta_p^2 = 0.63$. However, as with the MK-801 challenges, there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.48$, $p = .499$, $\eta_p^2 = 0.03$ and no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.57$, $p = .910$, $\eta_p^2 = 0.04$ (Figure 19 A). The 1.0 mg/kg amphetamine challenge resulted in a statistically significant increase in locomotor activity over time, $F(17, 238) = 7.34$, $p < .001$, $\eta_p^2 = 0.34$, but no statistically significant differences between groups, $F(1, 14) = 1.35$, $p = .265$, $\eta_p^2 = 0.09$, or significant time by treatment interactions, $F(17, 238) = 0.37$, $p = .990$, $\eta_p^2 = 0.03$ (Figure 19 B).

Adult males.

MK-801-induced locomotor activity. A similar pattern of results was observed in adult rats whereby locomotor activity significantly habituated following the saline injection, $F(17, 238) = 9.05$, $p < .001$, $\eta_p^2 = 0.40$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.02$, $p = .962$, $\eta_p^2 < 0.001$, and

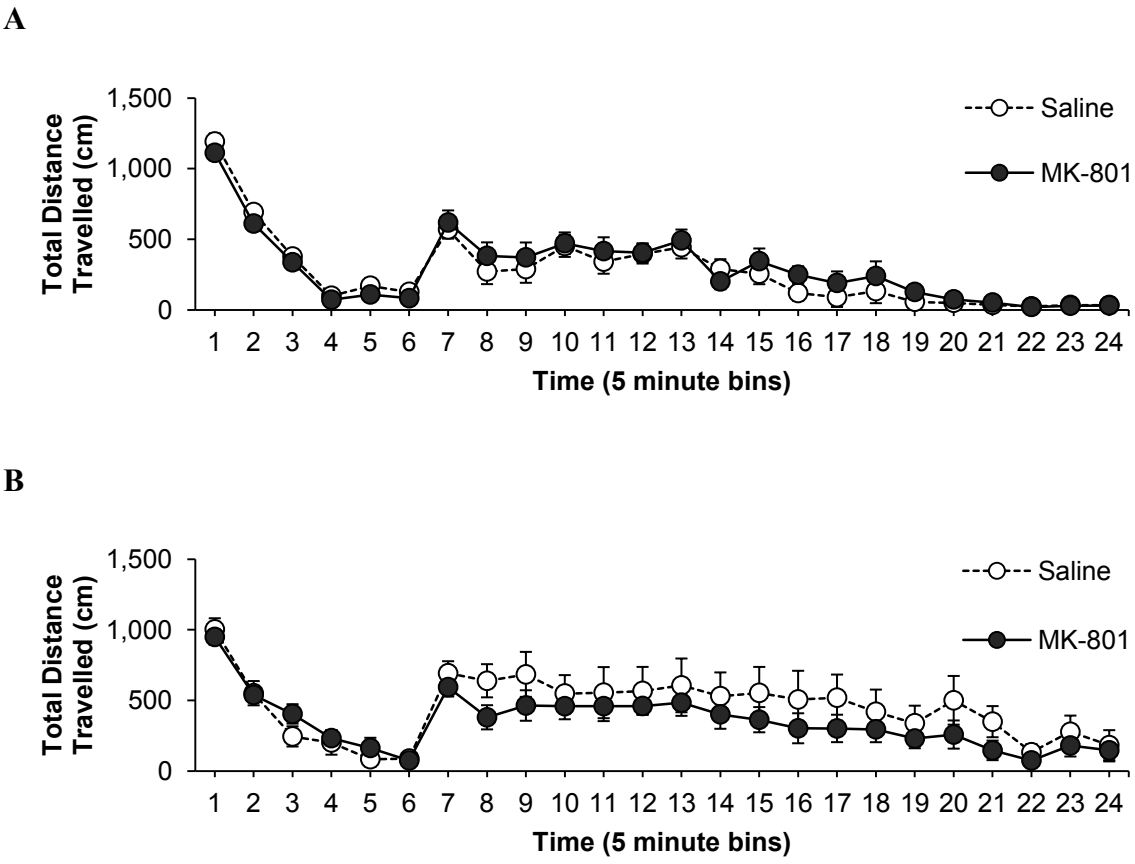


Figure 19. Mean (±SEM) total distance travelled (cm) by juvenile prenatal Saline and MK-801 male rats following a (A) 0.75 mg/kg amphetamine injection and (B) 1.0 mg/kg amphetamine injection.

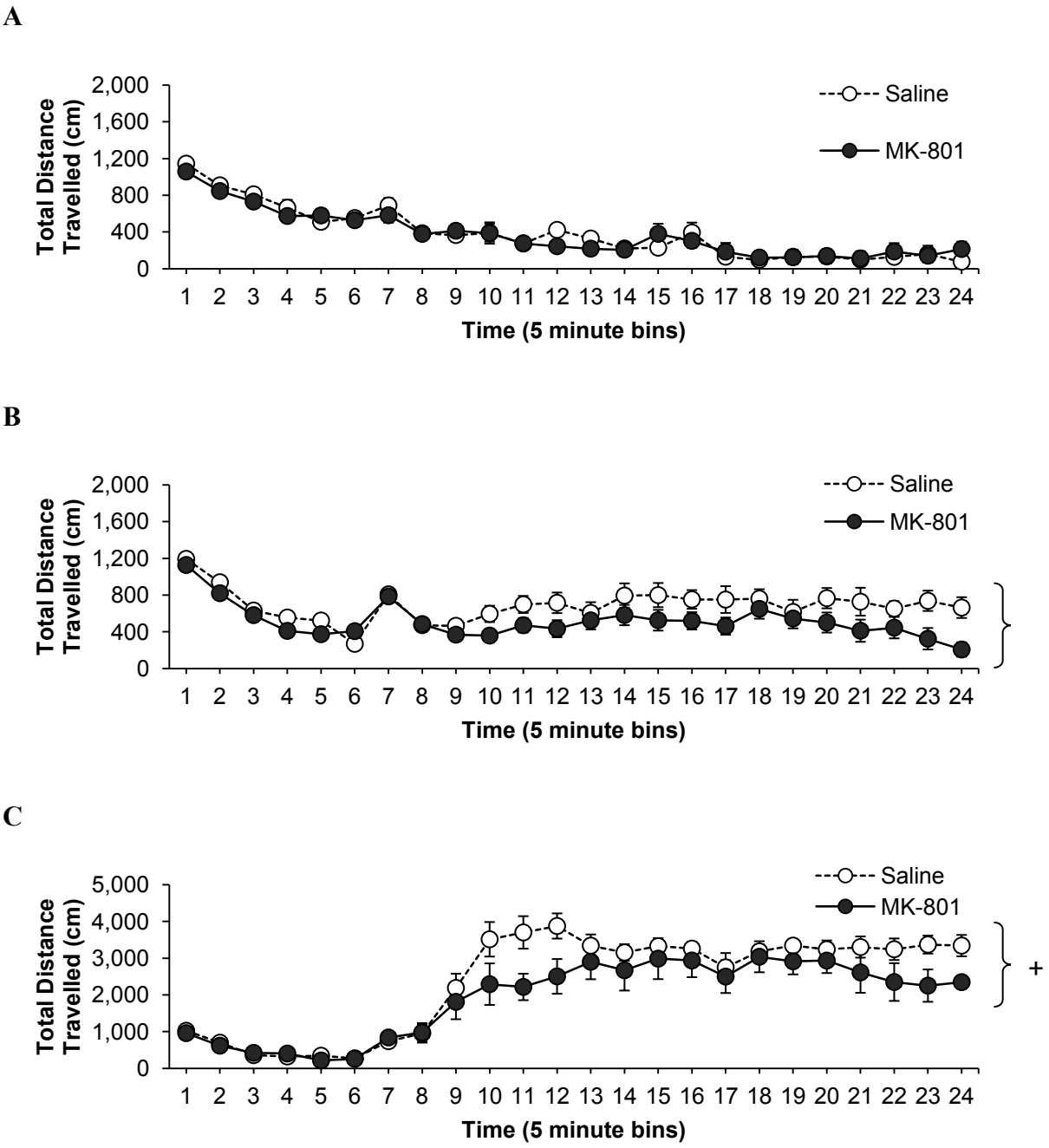


Figure 20. Mean (±SEM) total distance travelled (cm) by adult prenatal Saline and MK-801 male rats following a (A) saline injection, (B) 0.1 mg/kg MK-801 injection, and (C) 0.4 mg/kg MK-801 injection. * $p < 0.05$. $^{+}$ $p < 0.07$.

no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.72$, $p = .780$, $\eta_p^2 = 0.05$, (Figure 20 A). The 0.1 mg/kg MK-801 challenge also resulted in a statistically significant increase in locomotor activity over time, $F(17, 238) = 3.12$, $p < .001$, $\eta_p^2 = 0.18$. Importantly, MK rats displayed significantly lower locomotor activity post-injection compared to SAL rats, *prenatal treatment*: $F(1, 14) = 4.61$, $p = .050$, $\eta_p^2 = 0.25$. There was no statistically significant *time* by *prenatal treatment* interaction, $F(17, 238) = 1.35$, $p = .163$, $\eta_p^2 = 0.09$ (Figure 20 B). The 0.4 mg/kg MK-801 challenge also induced a statistically significant increase in locomotor activity over time, $F(17, 170) = 11.37$, $p < .001$, $\eta_p^2 = 0.53$. As was the case with the low dose of MK-801, MK rats appeared to move less than SAL rats in response to a high dose of MK-801, though this difference was at trend-level only, *prenatal treatment:* $F(1, 10) = 4.07$, $p = .071$, $\eta_p^2 = 0.29$. There was no significant *time* by *prenatal treatment* interaction, $F(17, 170) = 1.19$, $p = .278$, $\eta_p^2 = 0.11$ (Figure 20 C).

Amphetamine-induced locomotor activity. An injection of 0.75 mg/kg amphetamine resulted in a statistically significant increase in locomotor activity over time, $F(17, 221) = 8.79$, $p < .001$, $\eta_p^2 = 0.40$. However, there were no differences between MK and SAL rats in total distance travelled, *prenatal treatment:* $F(1, 13) = 0.84$, $p = .377$, $\eta_p^2 = 0.06$ and no significant *time* by *prenatal treatment* interaction, $F(17, 221) = 0.34$, $p = .994$, $\eta_p^2 = 0.03$ (Figure 21 A). A 1.0 mg/kg amphetamine challenge resulted in significantly increased locomotor activity over time, $F(17, 238) = 18.55$, $p < .001$, $\eta_p^2 = 0.57$. Again, however, there was no statistically significant difference between treatment conditions, *prenatal treatment:* $F(1, 14) = 0.88$, $p = .363$, $\eta_p^2 = 0.06$ or *time* by *prenatal treatment* interaction, $F(17, 238) = 0.89$, $p = .587$, $\eta_p^2 = 0.06$ (Figure 21 B).

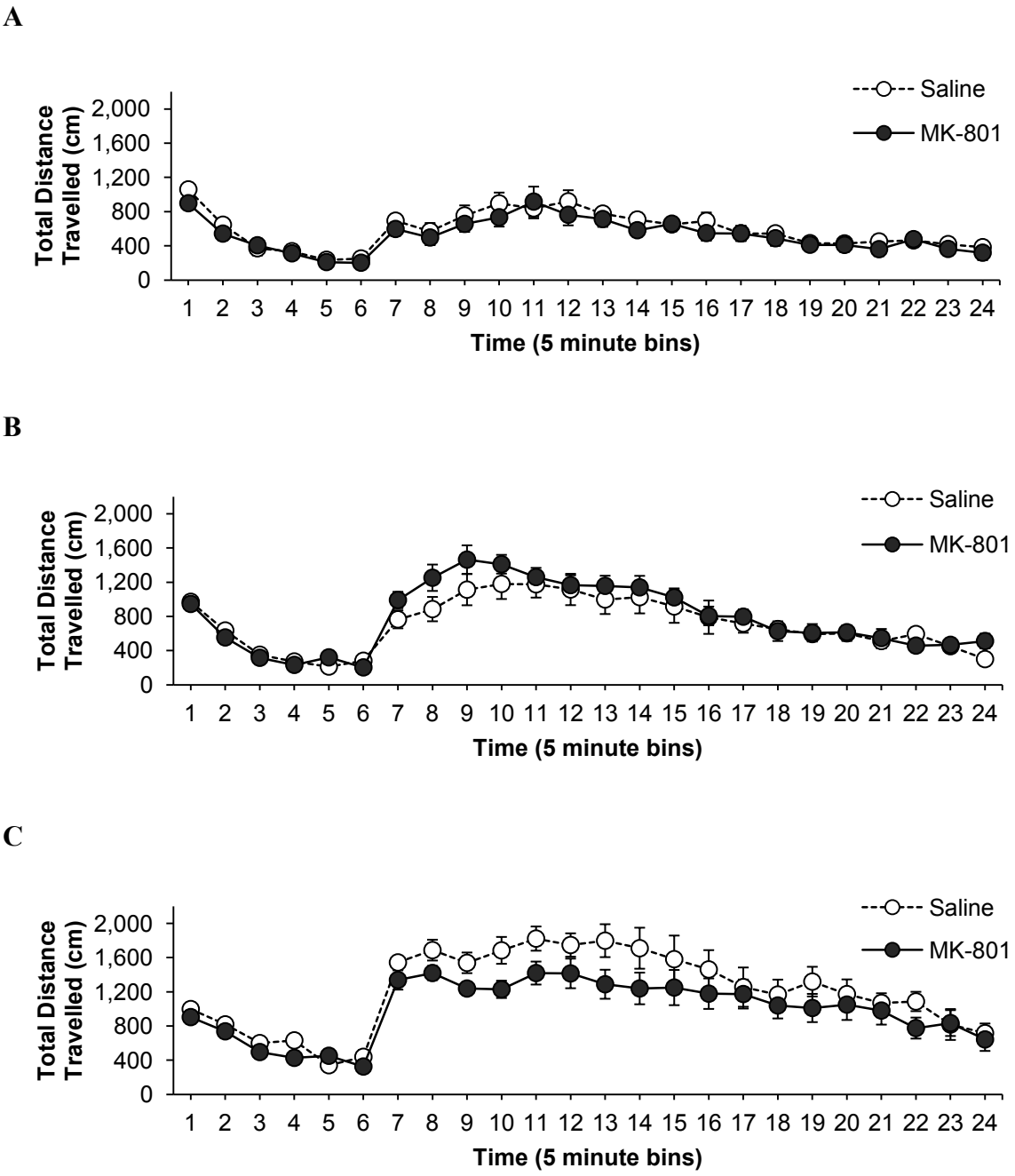


Figure 21. Mean (±SEM) total distance travelled (cm) by adult prenatal Saline and MK-801 male rats following a (A) 0.75 mg/kg amphetamine injection, (B) 1.0 mg/kg amphetamine injection and (C) 1.5 mg/kg amphetamine injection.

Following visual inspection of our data, we decided to challenge the adult males with an additional higher dose of 1.5 mg/kg amphetamine. In both prenatal treatment groups, rats displayed a statistically significant increase in locomotor activity over time following the injection, $F(17, 238) = 13.73$, $p < .001$, $\eta_p^2 = 0.50$. However, there were no statistically significant differences between MK and SAL rats, *prenatal treatment:* $F(1, 14) = 2.13$, $p = .166$, $\eta_p^2 = 0.13$, and no significant *time* by *prenatal treatment* interaction , $F(17, 238) = 1.04$, $p = .416$, $\eta_p^2 = 0.07$ (Figure 21 C).

Juvenile females

MK-801-induced locomotor activity. Following the saline injection (day 1), ambulatory locomotion significantly habituated over time $F(17, 221) = 5.73$, $p < .001$, $\eta_p^2 = 0.31$, but there was no statistically significant difference between the prenatal treatment groups, $F(1, 13) = 0.08$, $p = .778$, $\eta_p^2 = 0.00$ (Figure 22 A). There was no significant time by treatment interaction, $F(17, 221) = 0.66$, $p = .842$, $\eta_p^2 = 0.05$. The 0.1 mg/kg MK-801 challenge induced a statistically significant increase in locomotor activity over time, $F(17, 221) = 7.99$, $p < .001$, $\eta_p^2 = 0.38$. However, there was no statistically significant difference between prenatal treatment groups, $F(1, 13) = 0.04$, $p = .837$, $\eta_p^2 = 0.00$ and there was no significant *time* by *prenatal treatment* interaction, $F(17, 221) = 0.35$, $p = .993$, $\eta_p^2 = 0.03$ (Figure 22 B). The 0.2 mg/kg MK-801 challenge resulted in a statistically significant increase in locomotor activity over time**,** $F(17, 221) = 15.76$, $p < .001$, $\eta_p^2 = 0.55$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 13) = 0.01$, $p = .924$, $\eta_p^2 = 0.00$ and no significant *time* by *prenatal treatment* interaction, $F(17, 221) = 0.22$, $p = 1.00$, $\eta_p^2 = 0.02$ (Figure 22 C).

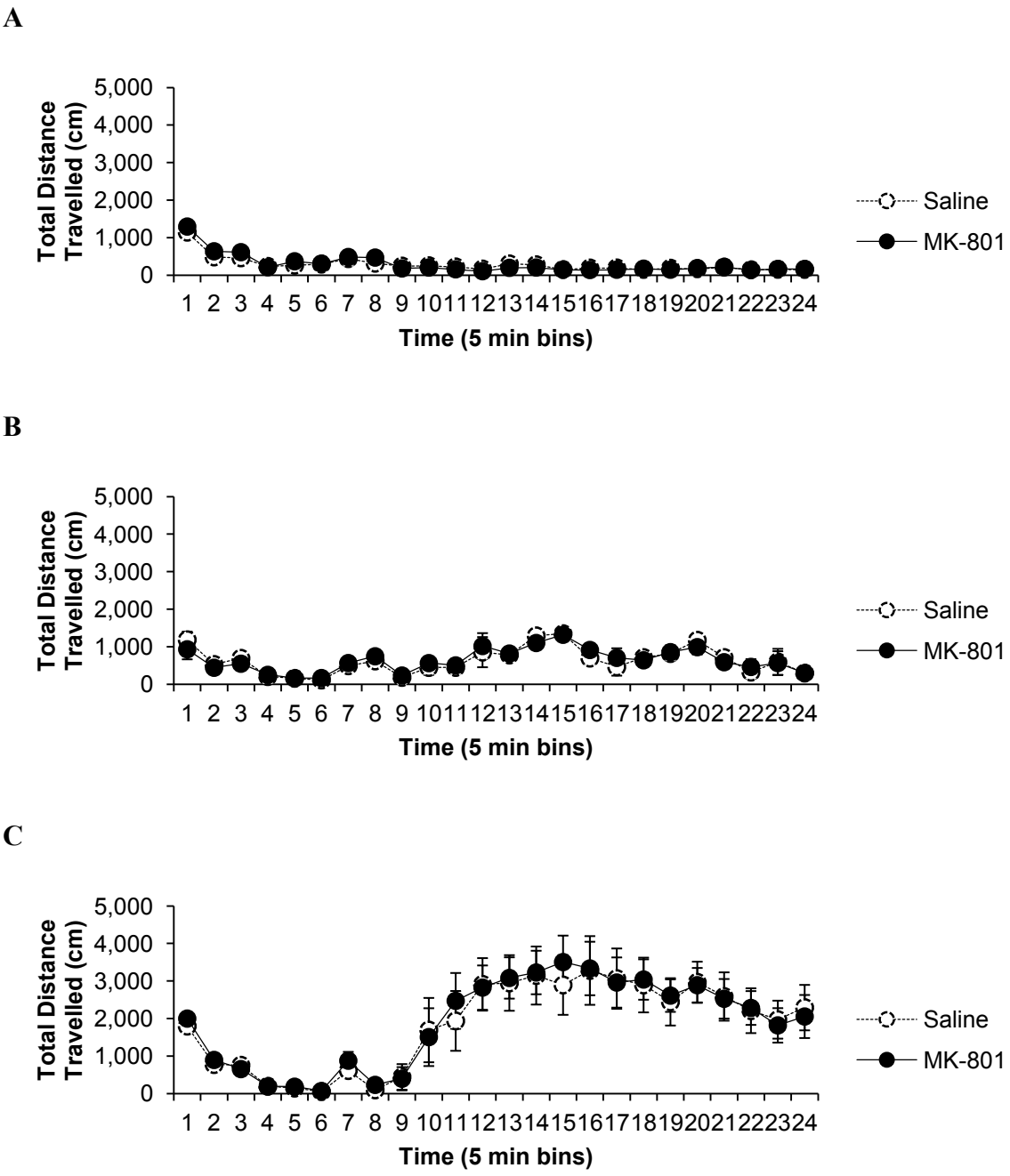


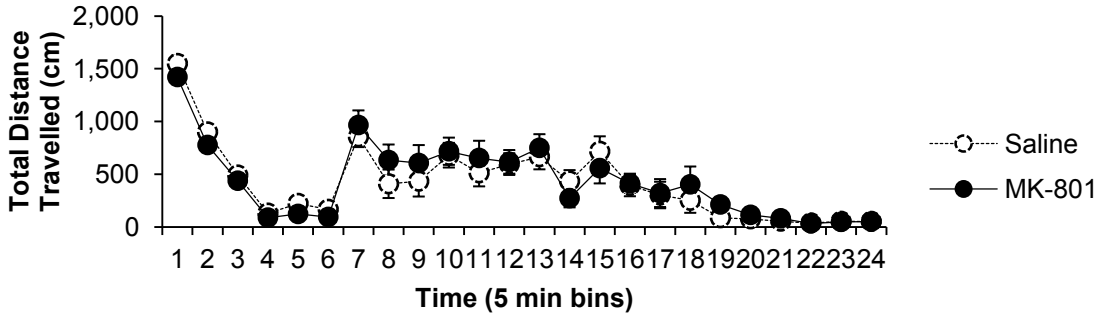
Figure 22. Mean (±SEM) total distance travelled (cm) by juvenile prenatal Saline and MK-801 female rats following a (A) saline injection, (B) 0.1 mg/kg MK-801 injection, and (C) 0.2 mg/kg MK-801 injection.

Amphetamine-induced locomotor activity. Challenge with a 0.75 mg/kg amphetamine dose resulted in a statistically significant increase in locomotor activity over time, $F(17, 221) = 21.73$, $p < .001$, $\eta_p^2 = 0.63$. However, as with the MK-801 challenges, there was no statistically significant difference between prenatal treatment groups, $F(1, 13) = 0.21$, $p = .656$, $\eta_p^2 = 0.02$ and no significant *time* by *prenatal treatment* interaction, $F(17, 221) = 76$, $p = .742$, $\eta_p^2 = 0.06$ (Figure 23 A). A 1.0 mg/kg amphetamine challenge resulted in a statistically significant increase in locomotor activity over time, $F(17, 221) = 7.80$, $p < .001$, $\eta_p^2 = 0.38$, but no statistically significant differences between prenatal treatment groups, $F(1, 13) = 0.02$, $p = .895$, $\eta_p^2 = 0.00$, or significant *time* by *prenatal treatment* interactions, $F(17, 221) = 0.86$, $p = .625$, $\eta_p^2 = 0.38$ (Figure 23 B).

Adult females.

MK-801-induced locomotor activity. As was the case in juvenile female rats, adult rat's locomotor activity significantly habituated following the saline injection, $F(17, 238) = 9.94$, $p < .001$, $\eta_p^2 = 0.42$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.02$, $p = .891$, $\eta_p^2 < 0.001$, and no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.96$, $p = .500$, $\eta_p^2 = 0.06$ (Figure 24 A). The 0.05 mg/kg MK-801challenge resulted in a statistically significant increase in locomotor activity over time, $F(17, 221) = 8.72$, $p < .001$, $\eta_p^2 = 0.40$. There was no statistically significant difference between prenatal treatment groups, $F(1, 13) = 1.01$, $p = .333$, $\eta_p^2 = 0.07$, or *time* by *prenatal treatment* interaction, $F(17, 221) = 0.93$, $p = .544$, $\eta_p^2 = 0.07$ (Figure 24 B). The 0.1 mg/kg MK-801 challenge also induced a statistically significant increase in locomotor activity over time, $F(17, 238) = 57.74$, $p <$

A



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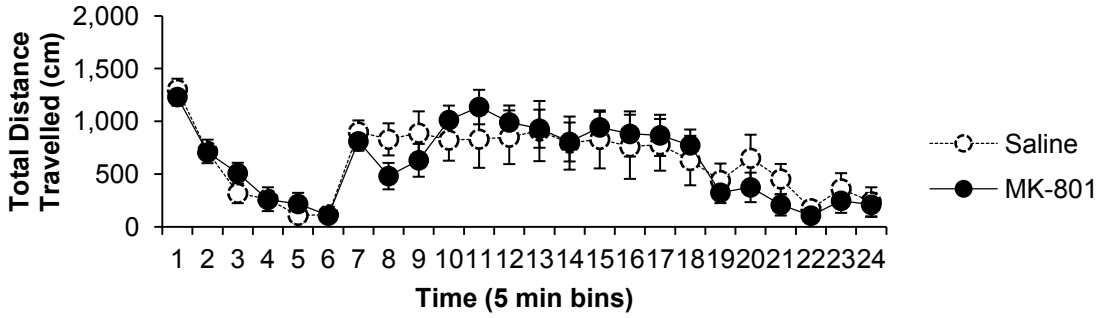


Figure 23. Mean (±SEM) total distance travelled (cm) by juvenile prenatal Saline and MK-801 female rats following a (A) 0.75 mg/kg amphetamine injection and (B) 1.0 mg/kg amphetamine injection.

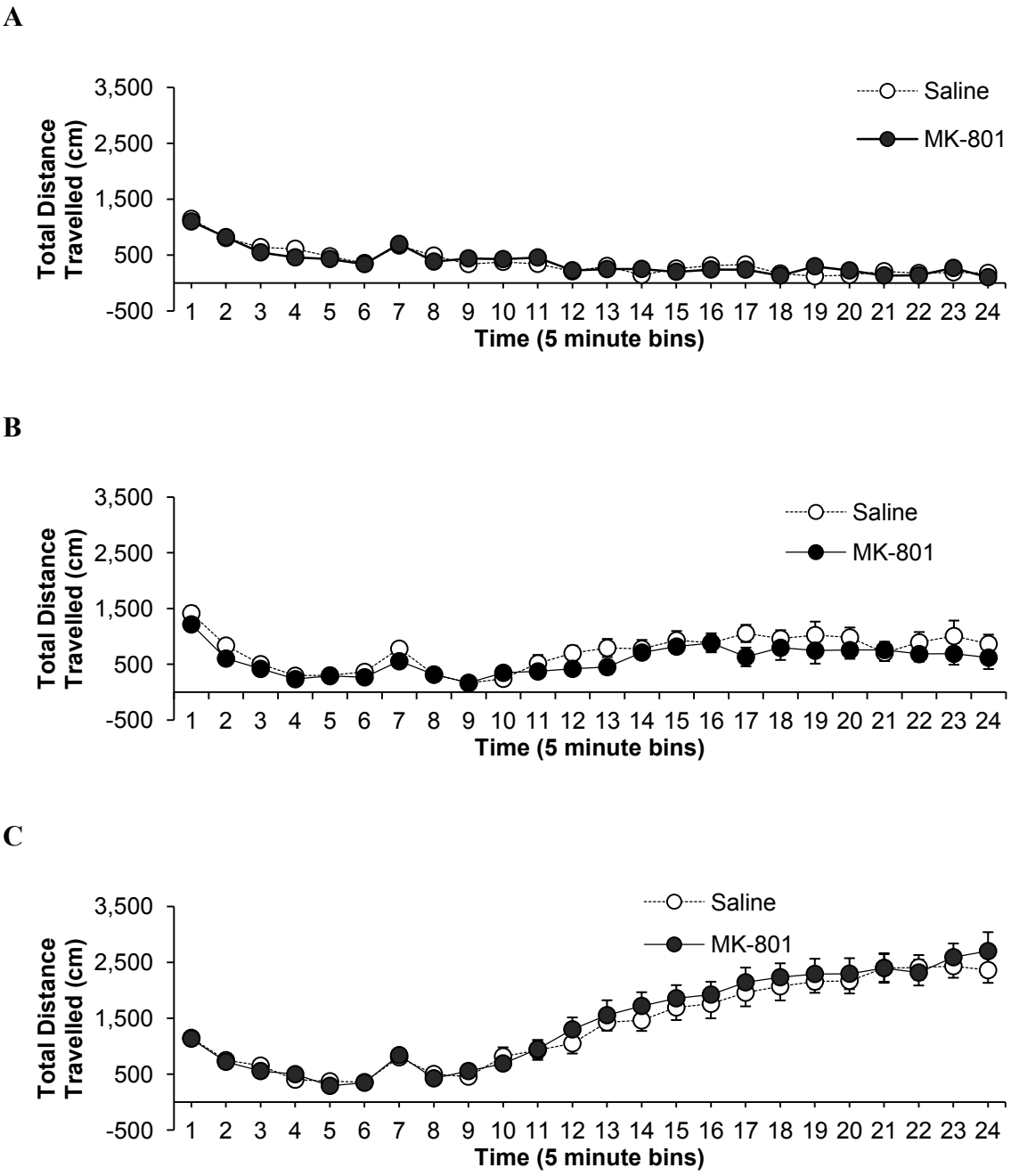


Figure 24. Mean (±SEM) total distance travelled (cm) by adult prenatal Saline and MK-801 female rats following a (A) saline injection, (B) 0.05 mg/kg MK-801 injection, and (C) 0.1 mg/kg MK-801 injection.

.001, $\eta_p^2 = 08$. There was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 2.02$, $p = .660$, $\eta_p^2 = 0.02$, or *time* by *prenatal treatment* interaction, $F(17, 238) = 0.448$, $p = .972$, $\eta_p^2 = 0.03$ (Figure 24 C).

Amphetamine-induced locomotor activity. Locomotor activity significantly habituated following the saline injection, $F(17, 238) = 6.96$, $p < .001$, $\eta_p^2 = 0.33$, but there was no statistically significant difference between prenatal treatment groups, $F(1, 14) = 0.07$, $p = .794$, $\eta_p^2 < 0.001$, and no significant *time* by *prenatal treatment* interaction, $F(17, 238) = 0.47$, $p = .964$, $\eta_p^2 = 0.03$ (Figure 25 A). An injection of 0.75 mg/kg amphetamine resulted in a statistically significant increase in locomotor activity over time, $F(17, 221) = 8.61$, $p < .001$, $\eta_p^2 = 0.40$. However, there were no differences between MK and SAL rats in total distance travelled, *prenatal treatment:* $F(1, 13) = 0.03$, $p = .377$, $\eta_p^2 < 0.001$ and no significant *time* by *prenatal treatment* interaction, $F(17, 221) = 0.41$, $p = .982$, $\eta_p^2 = 0.03$ (Figure 25 B). A 1.0 mg/kg amphetamine challenge resulted in significantly increased locomotor activity over time, $F(17, 238) = 24.11$, $p < .001$, $\eta_p^2 = 0.63$. Again, however, there was no statistically significant difference between prenatal treatment conditions, $F(1, 14) = 0.28$, $p = .603$, $\eta_p^2 = 0.02$ or *time* by *prenatal treatment* interaction, $F(17, 238) = 0.53$, $p = .934$, $\eta_p^2 = 0.04$ (Figure 25 C).

Delayed Non-Match to Place Task

As seen in Figure 26, following a 5 min delay, both the SAL, $t(6) = 4.86$, $p = .003$, $d = 1.84$, and MK rats, $t(7) = 5.92$, $p = .001$, $d = 2.09$, performed significantly above chancel level suggesting that the maternal manipulation did not induce working memory deficits at this delay. The 15 min delay also failed to induce deficits in SAL, $t(6) = 3.87$, $p = .003$, $d = 1.46$, or MK rats, $t(7) = 3.67$, $p = .008$, $d = 1.30$. The same was observed

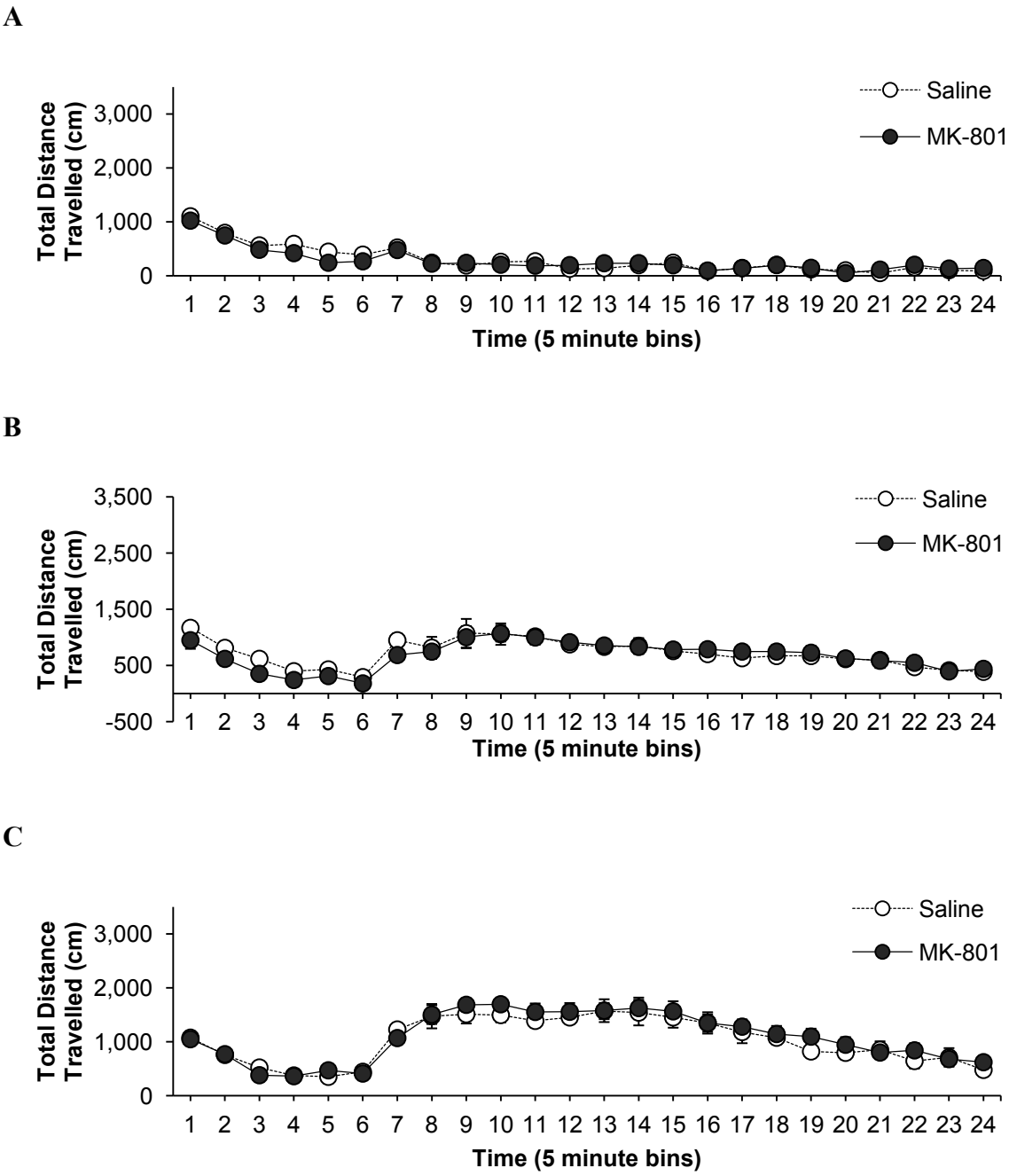


Figure 25. Mean (±SEM) total distance travelled (cm) by adult prenatal Saline and MK-801 female rats following a (A) saline injection, (B) 0.75 mg/kg amphetamine injection and (C) 1.0 mg/kg amphetamine injection.

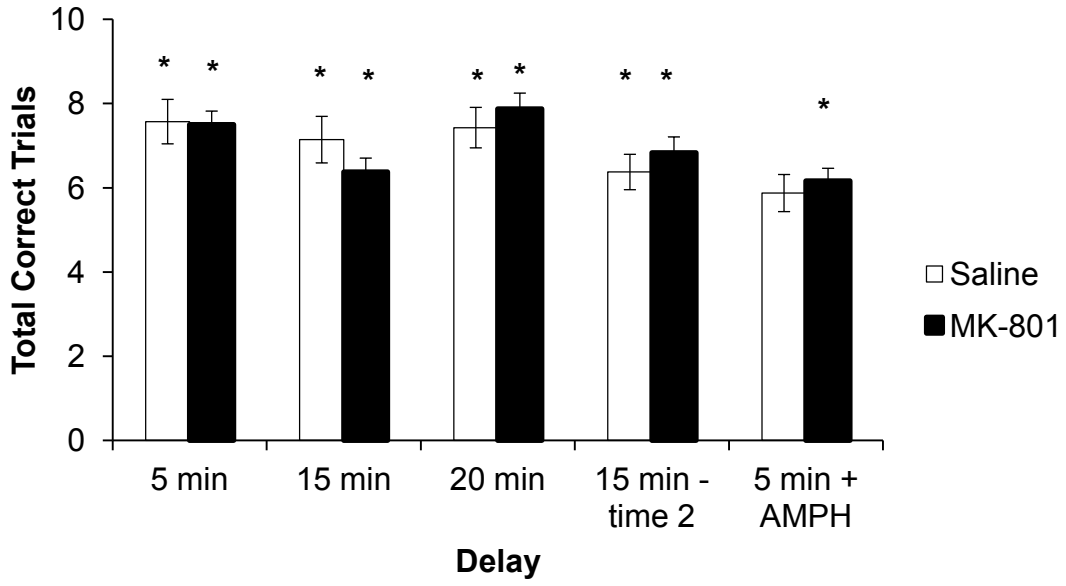


Figure 26. Mean (±SEM) correct trials for adult prenatal Saline and MK-801 rats in the delayed non-match to place task. Significantly different from chance (5), $p < .05$.

following a 20 min delay where both SAL, $t(6) = 5.05$, $p = .002$, $d = 1.91$, and MK rats, $t(7) = 4.95$, $p = .002$, $d = 1.75$, continued to perform significantly above chance level. Twenty-one days later, rats were retested following a 15 min delay and both SAL, $t(7) = 3.27$, $p = .014$, $d = 1.16$, and MK rats, $t(5) = 2.60$, $p = .048$, $d = 1.06$, performed above chance level. When rats were tested with a 5 min delay following a 0.75 mg/kg amphetamine injection, SAL rats, $t(7) = 1.99$, $p = .087$, $d = 0.70$, did not perform above chance level suggesting that the drug challenge impaired working memory. However, MK rats continued to perform above chance level following this same drug challenge, $t(5) = 2.91$, $p = .034$, $d = 1.19$.

Social Interaction

As depicted in Figure 27, MK rats spent less time sniffing the conspecific compared to SAL rats. This difference did not reach statistical significance, $t(14) = 1.19$, $p = .254$, but was supported by a large effect size ($g = 1.70$). Interestingly, MK rats spent more time wrestling the conspecific compared to SAL rats, though this difference was again not statistically significant, $t(14) = 1.38$, $p = .190$, $g = 1.90$. There were no statistically significant group differences in time spent following, $t(14) = 0.19$, $p = .854$, $g = 0.26$, or mounting the conspecific, $t(14) = 0.48$, $p = .640$, $g = 0.66$.

Sucrose Preference

There were no statistically significant differences between SAL and MK rats in total fluid consumption (tap water plus sucrose solution) on any of the 6 tests (Figure 28).

Water restricted tests. As seen in Figure 29 A, during 15 min access to the 1% sucrose solution under water-restricted conditions, MK rats preferred the sucrose solution over the tap water, $t(9) = 3.28$, $p = .009$, $d = 1.04$. Surprisingly, SAL rats did not show

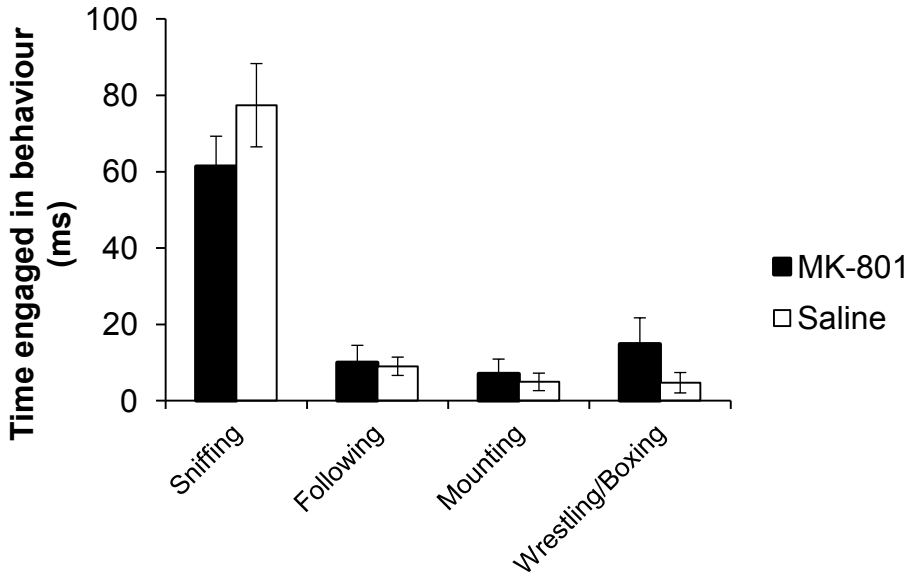
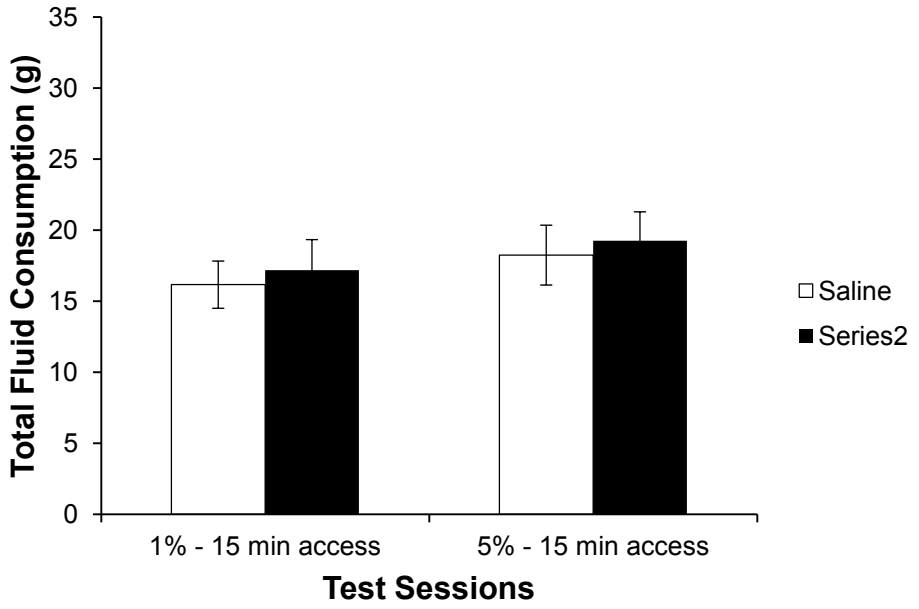


Figure 27. Mean (±SEM) time spent engaged in social behaviour during the 10 minute social interaction test in adult prenatal Saline and MK-801 rats.

A



B

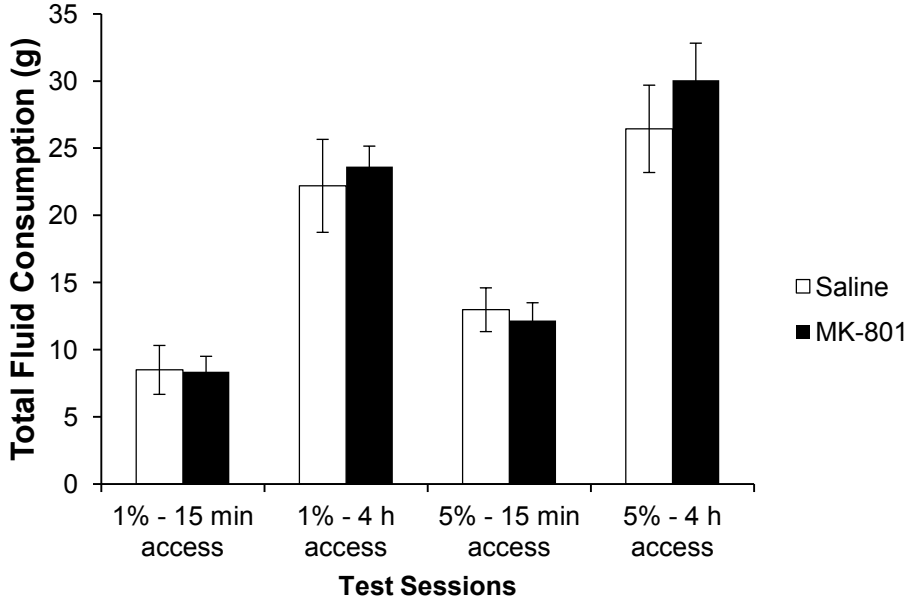
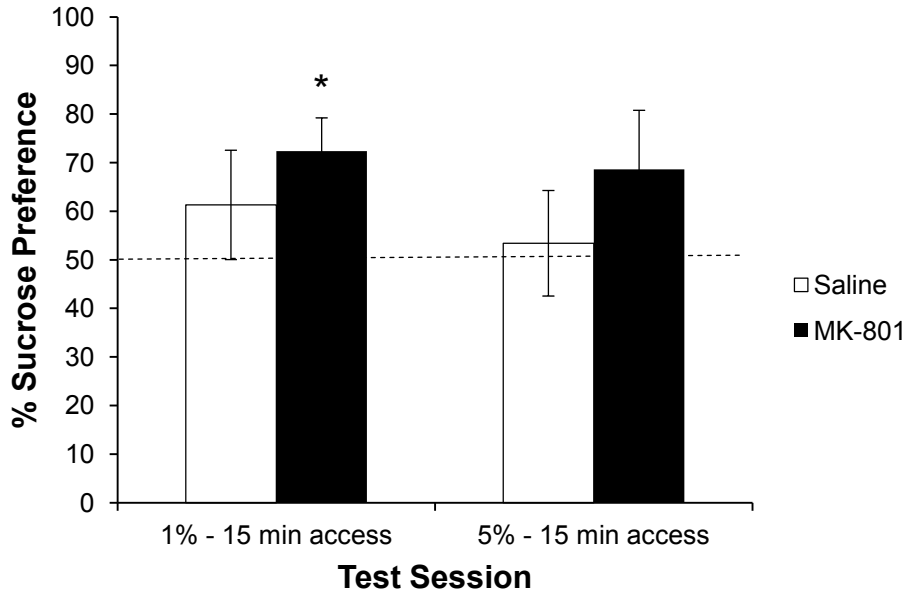


Figure 28. Mean (±SEM) total fluid consumption during sucrose preference tests in the adult prenatal Saline and MK-801 groups under (A) water-restricted conditions and (B) non-restricted conditions.

A



B

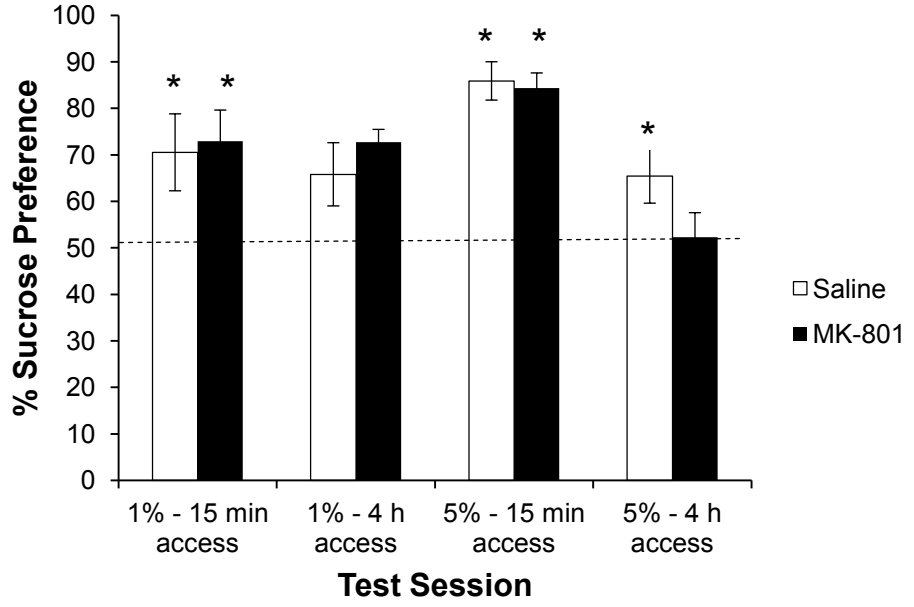


Figure 29. Mean (±SEM) percentage sucrose preference during sucrose preference tests under (A) water-restricted conditions and (B) non-restricted conditions in the adult prenatal Saline and MK-801 groups. *Significantly different from .5, $p < .05$

statistically significant preference for the 1% sucrose solution, $t(9) = 1.00$, $p = .342$, $d = 0.32$. When tested with a 5% sucrose solution, neither the MK or SAL rats showed sucrose or tap water preference during a 15 min test under water-restricted conditions, MK: $t(9) = 1.54$, $p = .159$, $d = 0.49$; SAL: $t(9) = 0.314$, $p = .761$, $d = 0.10$.

Non-restricted tests. As seen in Figure 29 B, under non-restricted conditions, both the MK and SAL rats showed statistically significant preference for the 1% sucrose solution during the first 15 min, MK: $t(9) = 3.41$, $p = .009$, $d = 1.08$; SAL: $t(9) = 2.48$, $p = .035$, $d = 0.79$. This preference was also observed in SAL and MK rats during a 4 hr test, MK: $t(9) = 8.26$, $p = .000$, $d = 2.61$; SAL: $t(9) = 2.33$, $p = .045$, $d = 0.74$. When tested with a 5% sucrose solution under non-restricted conditions, both the SAL and the MK rats preferred the sucrose solution over the tap water during the first 15 min, MK: $t(9) = 10.63$, $p = .000$, $d = 3.36$; SAL: $t(9) = 8.725$, $p = .000$, $d = 2.76$. Interestingly, during the 4 h test, although the SAL rats continued to show significant preference for the sucrose solution, $t(9) = 2.65$, $p = .026$, $d = 0.84$, the MK rats did not show preference for the sucrose solution over the tap water, $t(9) = 0.44$, $p = .674$, $d = 0.14$.

Set-Shifting

Pre-shift trials.

Acquisition. There was no statistically significant difference in the number of trials required to reach the first probe test between SAL ($M = 82.86$, $SD = 61.80$) and MK rats ($M = 83.75$, $SD = 57.03$); $t(13) = 0.03$, $p = .977$, $g = 0.02$.

Criterion. There was no statistically significant difference in the number of trials performed until a correct response in the probe test was achieved between the SAL ($M =$

84.47, *SD* = 64.53) and the MK rats (*M* = 92.50, *SD* = 66.97); *t*(13) = 0.24, *p* = .816, *g* = 0.12.

Probe. There was no statistically significant difference in the number of probe trials until a correct response was obtained between the SAL (*M* = 1.14, *SD* = 0.38) and the MK rats (*M* = 1.63, *SD* = 0.74); *t*(10.66) = 1.61, *p* =.136, *g* = 0.80.

Null trials. There was no statistically significant difference in the number of non-completed trials between the SAL (*M* = 9.14, *SD* = 17.58) and the MK rats (*M* = 4.13, *SD* = 7.24); *t*(13) = 0.74, *p* = .471, *g* = 0.38.

Taken together, these results suggest that prenatal exposure to MK-801 did not cause changes in the offspring's ability to learn and carry out the task.

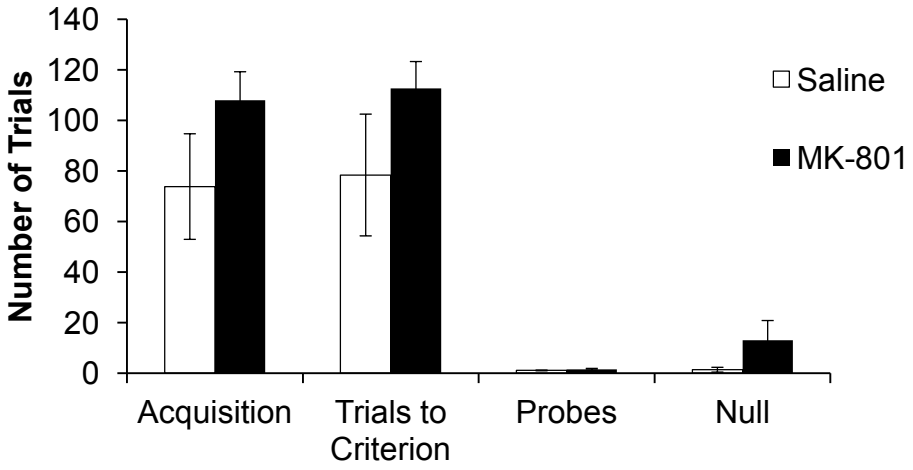
Post-shift trials.

Acquisition. As seen in Figure 30 A, MK rats required more trials to reach criterion compared to the SAL rats. This difference did not reach statistical significance, *t*(9.33) = 1.44, *p* = .183, but visual inspection of figure 30 A as well as consideration of the effect size (*g* = 0.77) suggest that the MK rats experienced difficulty acquiring the new rule.

Criterion. Rats from the MK group required more trials until the criterion was reached compared to the SAL rats (Figure 30 A). Again, this difference was not statistically significant, *t*(8.32) = 1.30, *p* =.229, but became apparent after visual inspection of Figure 30 A and a considerable effect size (*g* = 0.70).

Probe. There was no statistically significant difference in the number of probe trials between the SAL group and the MK group, *t*(9.57) = 1.36, *p* = .204, *g* = 0.67 (Figure 30 A).

A



B

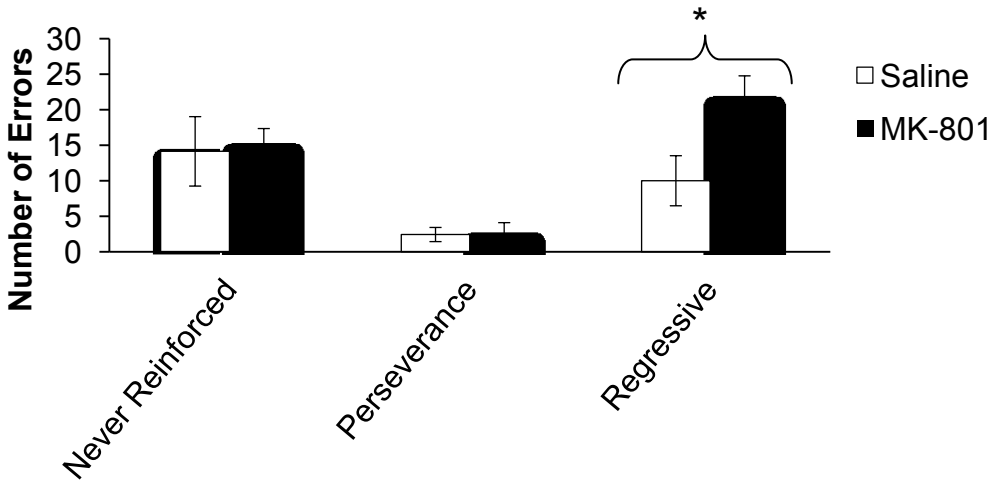


Figure 30. Mean (±SEM) (A) number of trials and (B) number of errors during the post-shift phase of the set-shifting task in the adult prenatal Saline and MK-801 groups. *Significantly different, $p < .05$.

Null trials. There was no statistically significant difference in the number of trials performed between the SAL group and the MK group, $t(7.21) = 1.50$, $p = .177$, $g = 0.72$ (Figure 30 A).

Errors. As seen in figure 30 B, there was no statistically significant difference in the number of never-reinforced errors between the SAL group and the MK group , $t(8.70) = 0.16$, $p = .878$, $g = 0.09$. There was no statistically significant difference in the number of perseverative errors between the SAL rats and the MK rats, $t(13) = 0.04$, $p = .971$, $g = 0.02$. Interestingly, the MK rats made significantly more regressive errors compared to the SAL rats, $t(13) = 2.47$, $p = .028$, $g = 1.20$. This difference suggests that MK rats experienced difficulty maintaining a newly acquired rule and had the tendency to revert back to the previous rule.

Dopamine/DOPAC ratio

As seen in Figure 31, although no between-group differences reached statistical significance, medium effect sizes suggested that DA turnover appeared to be lower in MK rats compared to SAL rats in the NAcc, $t(10.11) = 1.40$, $p = .193$, $g = 0.70$, dmSTR, $t(7.86) = 1.60$, $p = .149$, $g = 0.80$, and vPFC, $t(14) = 1.40$, $p = .185$, $g = 0.70$, following a 0.75 mg/kg amphetamine injection. The difference did not appear to be as large in the dlSTR, $t(13) = 0.90$, $p = .387$, $g = 0.46$.

Summary

No statistically significant differences were found between SAL and MK dams on any of the assessed maternal behaviours suggesting that treatment condition did not impact the way in which dams cared for their offspring during the first 14 days following parturition. Therefore, we assume that any observed behavioural differences between

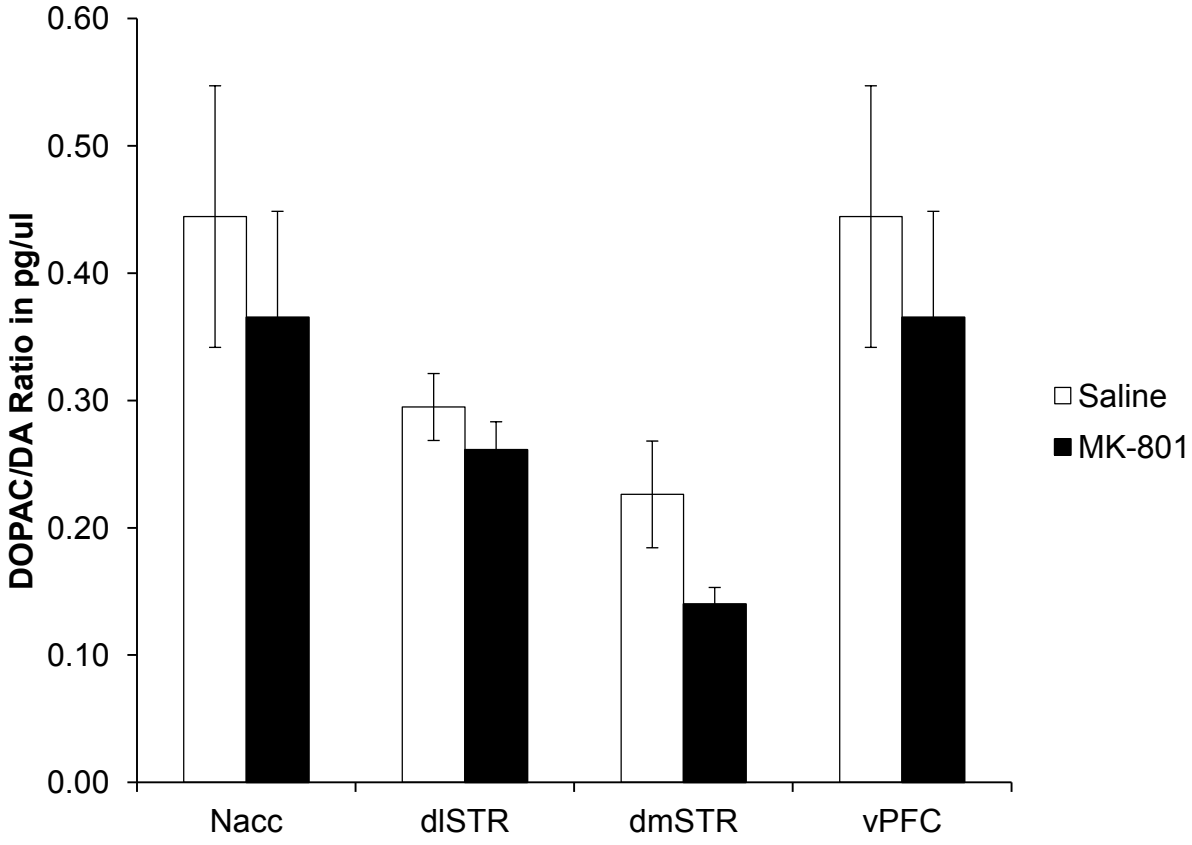


Figure 31. Mean (±SEM) DOPAC/DA ratio in the nucleus accumbens, dorsal lateral striatum, dorsal medial striatum, and ventral prefrontal cortex in the adult prenatal Saline and MK-801 groups.

SAL and MK offspring were not the result of differences in maternal care. Our hypothesis that MK offspring would have lower weights compared to SAL offspring was not supported for the males, with no statistically significant differences found at PND 2, 7, 14, or 21. However, we found that MK females weighed significantly less than SAL females at PND 2.

Hypotheses regarding NOP in male rats were partially supported. During the juvenile period, neither the SAL or MK rats showed statistically significant NOP following the 90 sec and 4 h delays, but both groups showed statistically significant NOP following a 15 min delay. Interestingly, while both the SAL and MK adult males showed statistically significant NOP after a 15 min delay, NOP was only observed in the SAL rats following the 90 min delay, suggesting that prenatal MK-801 administration led to altered NOP following this delay. Neither the SAL nor MK adult males showed statistically significant NOP following a 4 h delay suggesting that they may not have recognized the sample object after this longer delay. A different pattern of results was observed with female rats. Following a 90 sec delay, juvenile SAL females showed statistically significant NOP but the MK rats did not, while the exact opposite was observed following the 15 min delay. Consistent with what was found with males, neither the SAL or MK juvenile females showed statistically significant NOP following a 4 h delay. In adulthood, MK females showed NOP following the 90 min delay while the SAL rats did not. NOP was not observed in either of the groups following a 4 h delay.

Our hypotheses regarding AMPH and MK-801-induced locomotor activity in juvenile male rats were supported. No statistically significant differences were found in locomotor activity between SAL and MK rats following any of the AMPH or MK-801

challenges. In adult male rats, we found statistically significant differences between SAL and MK rats following the MK-801 challenges, but these differences were contrary to our hypotheses. More specifically, we observed reduced responsiveness to MK-801 in the MK males compared to the SAL males. No statistically significant differences were observed in response to the AMPH challenges. As was the case with juvenile males, there were no differences between SAL and MK females in locomotor activity following any of the drug challenges during the juvenile period. Contrary to our hypothesis, we did not observe any statistically significant differences between SAL and MK adult females following the MK-801 or AMPH challenges.

Our hypotheses about working memory were not supported. More specifically, all adult male rats were successful in completing the delayed non-match to place task at all delays and no statistically significant differences between SAL and MK rats were observed. These results suggest that prenatal MK-801 administration did not induce deficits in working memory as assessed with this task.

When assessing for differences in social behaviour in adult male offspring, we found that MK rats spent less time sniffing and more time wrestling the conspecific compared to SAL rats. In line with our hypothesis, these results suggest a possible tendency for MK offspring to spend less time engaged in prosocial behaviours. However, we did not see any statistically significant differences between SAL and MK rats in time spent following and mounting the conspecific.

In the sucrose preference test, we hypothesized that MK rats would not show preference for sweetened water, indicating anhedonia-like behaviour. This hypothesis was partially supported. When rats were given 4 h access to a 5% sucrose solution under

ad libitum water and food conditions, adult male SAL rats showed statistically significant preference for the sweetened water while the MK rats did not. This effect, however, was not observed during the first 15 min of the test, or when the rats were tested with a 1% sucrose solution. Furthermore, when rats were tested with a 1% sucrose solution under food and water restriction conditions, MK rats showed statistically significant preference for the sweetened water while the SAL rats did not. When tested under the same conditions with a 5% sucrose solution however, neither the SAL or MK rats showed statistically significant preference for the sweetened solution.

In the set-shifting task, we hypothesized that adult male MK rats would show an impaired ability to shift to a new rule. More specifically, we expected to see more perseverative errors in MK rats compared to SAL rats. These hypotheses were again only partially supported. Consistent with our hypothesis, there were no statistically significant differences between SAL and MK rats in the pre-shift phase suggesting that prenatal MK-801 administration did not result in impaired ability to learn the task. However, during the post-shift phase, MK rats appeared to take more time to acquire the new behaviour. Contrary to our hypothesis, we did not find differences between SAL and MK rats on number of perseverative errors committed. Interestingly however, we found that MK rats made significantly more regressive errors than the SAL rats suggesting that MK rats experienced difficulty maintaining a newly acquired rule.

Finally, we hypothesized that adult male MK offspring would show enhanced DA activity following an injection of AMPH. Although we did not observe any statistically significant differences, DA turnover appeared to be reduced in the NAcc, dmSTR, and

vPFC of MK rats compared to SAL rats, indicating that prenatal MK-801 administration may have resulted in a reduced DA response following an AMPH challenge.

General Discussion

The current thesis set out to investigate whether prenatal manipulations could induce behavioural and neurochemical changes that would be manifested in adult, but not juvenile male and female offspring. The prenatal manipulation in Chapter 1 was based on the epidemiological relation between maternal infection and schizophrenia in humans and involved exposure of pregnant females to poly I:C, a viral mimic, at day 15 of gestation. Behaviour was assessed in the juvenile and adult offspring of these dams. In Chapter 2, we set out to explore the role of the NMDA receptor in subsequent neurodevelopment by exposing pregnant females to repeated administration of the NMDA receptor antagonist, MK-801. A battery of tasks designed to assess positive, negative, and cognitive-like behaviours were used in the juvenile and adult offspring.

Chapter 1: Behavioural consequences of prenatal immune challenge in the juvenile and adult rat offspring

Experiments in Chapter 1 aimed to replicate findings from numerous researchers suggesting that prenatal treatment with poly I:C induces deficits in cognitive tasks, attention deficits, and hypersensitivity to the locomotor activating effects of psychostimulant drugs. Furthermore, we predicted that these behavioural alterations would be manifested in adult, but not juvenile offspring. Our results did not support the hypothesis that maternal infection via prenatal poly I:C administration produces schizophrenia-like symptoms in adult offspring, thereby putting into question the robustness of this commonly used model.

Effects of poly I:C on maternal weight change and immune response.

Maternal weight change has been proposed to serve as an indicator of the pregnant dam's

individual immune response to the poly I:C challenge (Meyer & Feldon, 2012). By administering exogenous cytokines to pregnant rats and mice, researchers have shown that elevated maternal cytokines, markers of the immune response, is sufficient in inducing behavioural effects in the offspring (Samuelsson et al., 2006; Smith et al., 2007). Thus, it has been proposed that the release of pro-inflammatory cytokines is the primary mechanism by which maternal poly I:C challenges induces its effects. Concomitantly, maternal weight loss is thought to serve as an index of the dam's immune response.

In the present study, no weight loss was observed on the day following the poly I:C injection and there were no differences between SAL and poly I:C dams in weight change following the treatment. It is thus possible that our prenatal challenge was not successful in evoking a maternal immune response that is thought to be crucial in inducing subsequent behavioural alterations in the offspring. Indeed, this may explain why we were unable to show behavioural deficits in adult poly I:C offspring. This potential explanation, however, assumes that maternal weight loss/change following poly I:C challenge does in fact reflect potency of the immune response which is in turn related to severity of the observed deficits in adult offspring. However, the literature on the subject of maternal weight change is inconsistent and researchers have yet to reach a consensus.

Previous research has described a significant variability in the maternal body weight response to poly I:C injection. According to Wolff and Bilkey (2010), however, maternal weight loss appears to be independent from the effect of maternal infection on foetal neurodevelopment. More specifically, they found that prenatal poly I:C challenge

induced deficits in PPI of the acoustic startle response in adult offspring and that these deficits were not correlated with maternal weight loss on the day following the maternal manipulation. These findings are contrary to previous reports of existing differences in behavioural outcome between offspring of dams that lost weight and offspring of dams that gained weight in a variety of behavioural tasks such as PPI, sensitivity to the locomotor-activating effects of DA agonists and NMDA receptor antagonists, and sucrose preference tests (Bronson, Ahlbrand, Horn, Kern, & Richtand, 2011; Vorhees et al., 2012).

A recent study by Missault et al. (2014) found that dams that lost weight following poly I:C challenge, but not those that gained weight, showed increased levels of TNF-α compared to control dams. Furthermore, offspring born to dams that had lost weight showed the most severe behavioural outcome while offspring born to poly I:C-treated dams that did not lose weight did not show clear behavioural deficits. Interestingly, they did see an effect for maternal weight loss on PPI in adult offspring. These mixed findings suggest that although there may be an association between maternal weight loss and subsequent behavioural deficits in the offspring, this association is complex and may be limited to specific behavioural tasks. In the context of our study, it is possible that the absence of a strong immune response, as indexed by the absence of maternal weight loss following the poly I:C injection, may explain our null findings.

One major limitation in our study was our failure to monitor the pregnant female's immune response following the poly I:C injection. Given the literature that seems to indicate a possible role for maternal weight loss/change following the maternal treatment, future studies should aim to follow the dam's febrile response more closely by

either weighing the females throughout gestation, monitoring changes in body temperature that would indicate febrile response, or collecting blood following the treatment in order to quantify the presence of important pro-inflammatory cytokines. Researchers may also wish to keep track of which offspring came from high immune response dams versus those born to low immune response dams in an attempt to, on one hand, evaluate the association between maternal immune response and behavioural impairment in the offspring and, on the other hand, to optimize their chances of seeing an effect if one is present.

Another important consideration that is generally not discussed in published articles describing the effects of maternal infection is that there are batch and lot variations in the pyrogenic and cytokinogenic activity of commercial poly I:C (L. Harvey & Boksa, 2012). In an effort to facilitate comparisons and replication, researchers are encouraged to carefully select dosages and routes of administration of poly I:C based on pilot studies with their own batches of poly I:C rather than relying on published studies that were carried out with different batches. In the present study, although we chose to not collect blood samples from our pregnant dams in an effort to reduce the risk of stress as a confound variable, we attempted to control for this limitation by conducting a pilot study. Non-pregnant female rats were injected with saline or poly I:C from two separate batches and of two different doses and plasma was assayed for IL-6, in order to make sure that cytokine titers are within the range reported in the literature. Nevertheless, because we did not quantify levels of IL-6 in our experimental dams, we cannot conclude with absolute confidence that IL-6 levels following our poly I:C injections were at a desired level.

Reports of individual variations in immune response as well as differences in batches of poly I:C illustrate that successful replication of poly I:C studies is all but trivial. In fact, the effects of this early manipulation are highly dependent on a number of complex factors that need to be carefully controlled.

Effects of poly I:C on NOP in juvenile and adult offspring. As previously discussed, patients with schizophrenia often present with impaired object recognition memory. Previous researchers that have used poly I:C to model maternal infection in rodents have shown alterations in NOP in adult, but not juvenile, offspring (W. Y. Li, Chang, Lee, & Lee, 2014; Lukasz et al., 2013; Ozawa et al., 2006). Thus, our first goal was to examine whether adult, but not juvenile, rats born to poly I:C treated dams would exhibit alterations in novel object preference following a series of delays. Our results from adult male rats show that both SAL and poly I:C offspring showed novel object preference following a 90 sec and 15 min delay, suggesting that prenatal infection did not result in differences in object recognition memory following these delays. Interestingly, neither the SAL nor poly I:C rats showed NOP at the longer 4 h delay, suggesting that they were unable to recognize the familiar object. In contrast to our hypothesis, however, this absence of novelty preference was not limited to the poly I:C rats suggesting that the rats failed to recognize the novel object due to difficulties unrelated to prenatal infection, as detained below. Interestingly, results from adult females were more in line with our hypothesis. Although both the SAL and poly I:C adult females showed NOP at the 90 sec and 15 min delays, poly I:C females no longer showed NOP following the 4 h delay while SAL females did. This suggests that prenatal exposure to poly I:C induced a deficit

in object recognition in adult female offspring that was only observable following the longer 4 h retention period.

An important limitation of the NOP task is that its usefulness as a test of recognition memory is limited to situations in which rats display NOP that is significantly above chance (Gaskin et al., 2010). In other words, when rats show statistically significant NOP, one can safely infer that the rats are able to recognize the familiar object. However, a lack of NOP may not necessarily indicate an impairment in the retrieval of the representation of the familiar object, as manipulations aimed at increasing the familiarity of the sample object (e.g. increasing time of familiarization phase) had no effect on the preference for the novel object during the retention test (Gaskin et al., 2010). Therefore, caution should be used when drawing conclusions about the reason rats fail to display significant NOP, as this may not always be a reliable sign of recognition memory impairment. With this in mind, it is possible that the absence of NOP following a 4 h delay in both the SAL and poly I:C adult male offspring does not imply that the rats did not recognize the sample object. In fact, NOP has been documented in adult Sprague-Dawley rats up to a 24 hour delay (Reger, Hovda, & Giza, 2009). One possible explanation for the lack of NOP following a 4 hour delay is that the sequence of delays used was not counterbalanced – all rats were first tested with a 90 sec delay, followed by a 15 min delay, and finally the 4 h delay. It is thus possible that rats were simply no longer interested in the task by the time they were tested with the longer delay.

The first and foremost hypothesis was that prenatal poly I:C would result in impaired NOP in adult rats. Secondly, we hypothesized that this impairment would not be observed in juvenile offspring. Because our adult hypothesis was not supported,

results from the juvenile rats are less relevant. Nonetheless, our juvenile results were unexpected and may have important ramifications for future studies. Since NOP was not detected in the saline-treated group in any of the tested delays, we were unable to directly test the secondary hypothesis. Rather, we observed that although poly I:C male offspring showed NOP following a 90 sec delay, the SAL rats did not. The difference in sample size between the SAL and poly I:C rats may partly explain why the difference was statistically significant for one group but not for the other. However, the small effect size for the SAL rats ($d = 0.46$) relative to that of the poly I:C rats ($d = 1.12$) suggests that this was not the case. Nevertheless, because our control group failed to show NOP at such a short delay, and neither the SAL or poly I:C rats showed NOP after the 15 min and 4 h delays, interpretation of this apparent enhancement of NOP should be made with caution. Finally, in female juvenile offspring neither the SAL or poly I:C rats showed NOP at any of the delays used.

Although the NOP test has been used to assess object recognition memory in adult rats born to poly I:C-treated dams, no researchers, to my knowledge, have used the task to assess the impact of prenatal poly I:C treatment on object recognition memory in juvenile offspring. There have been reports showing that rats aged from PND 18 to PND 31 have the ability to perform the NOP task with delays ranging from 1 min to 1 h between familiarization and test phase (Ainge & Langston, 2012; Anderson et al., 2004; Heyser & Ferris, 2013; Kruger, Brockmann, Salamon, Ittrich, & Hanganu-Opatz, 2012). Thus, it appears that the shorter delays used in the present experiment (90 sec and 15 min) were within the range that would allow expression of NOP in juvenile rats. In contrast, Anderson et al. (2004) report a significant impairment in NOP in juvenile rats

following a 120 min delay which may suggests that our use of a 4 h delay with juvenile rats may have been too ambitious.

Another potential explanation as to why NOP in juvenile rats was not observed in the present experiment is that the rats used in the experiments described in Chapter 1 were of the Wistar strain as opposed to Long-Evans rats that are more commonly used in behavioural experiments assessing memory. However, this explanation does not appear to be satisfying as NOP has been reported not only in juvenile Long-Evans (Anderson et al., 2004), but also in Sprague-Dawley (Heyser & Ferris, 2013) and Wistar rats (Kruger et al., 2012).

Another important consideration is that the apparatus used to test NOP in juvenile rats was the same square arena that has been optimized to test NOP in adult rats in our facility. Furthermore, although all pairs of objects used had previously been tested for the lack of innate preference in adult rats, they had not been tested with juvenile rats. Thus, it is possible that the use of an arena adapted for juvenile rats as well as objects pre-assessed for preference in juvenile rats may have yielded different results. Indeed, Heyser and Ferris (2012) report that PND 21 rats tested in a square arena, as was used in the present experiment, spent a significant amount of time in the corners which lead to increased between-subject variability and decreased performance in this task. The authors report that using a circular arena with rats of the same age significantly increased object exploration and decreased variability, making it a well suited task for young rats. It should be noted, however, that despite the fact that juvenile rats may be more likely to spend more time investigating objects in a circular arena as opposed to a square arena, it has been proposed that the amount of time spent investigating during the familiarization

phase is not a predictor of later performance in the retention test (Gaskin et al., 2010). In fact, Gaskin et al. (2010) suggest that significant preference for the novel object may only occur if a minimal amount of 60 to 90 sec of object investigation during familiarization is achieved. Beyond this minimal amount, however, additional sample object investigation does not appear to result in a significant increase in the magnitude of NOP. In the present experiment, rats exploring less than 60 sec during familiarization were excluded from the analyses.

Effects of poly I:C on AMPH-induced locomotor activity in juvenile and adult offspring. Unlike the cognitive and, to a certain extent, negative symptoms of schizophrenia, the positive symptoms represent a challenge as their subjective nature make them particularly difficult to model in animals. As previously discussed, exposure to the DA agonist, AMPH, has been shown to exacerbate positive symptoms in patients with schizophrenia and to increase mesolimbic DA activity. This can be modeled in rodents whereby an AMPH challenge increases mesolimbic DA activity and, consequently, enhances locomotor activity. Hyperactivity responses to AMPH in rodents is frequently used as a behavioural assessment serving as an index of underlying dopaminergic activity. Hypersensitivity to the locomotor-activating effects of AMPH has been repeatedly reported in adult offspring of both mice and rats born to poly I:C treated dams (Meyer, Nyffeler, Yee, et al., 2008; Ozawa et al., 2006; Smith et al., 2007; Vuillermot et al., 2010; Zuckerman & Weiner, 2005). Furthermore, Zuckerman et al. (2003) showed that the emergence of increased sensitivity to the locomotor-stimulating effects of AMPH were only observed in adult offspring.

Based on these findings, we hypothesized that rats born to poly I:C-treated dams would show enhanced locomotor activity following an injection of AMPH compared to SAL rats. Furthermore, we predicted that this enhanced sensitivity would be observed in adult but not juvenile rats. Our results do not support these hypotheses. In fact, no differences were found between prenatal treatment conditions in adult male rats suggesting that prenatal exposure to poly I:C did not result in mesolimbic DA hyperactivity. Interestingly, poly I:C adult females showed lower locomotor responses to the low dose of AMPH compared to the SAL adult females.

Our adult results were surprising given the overwhelming reports of AMPH hypersensitivity in poly I:C-treated offspring. However, a close examination of the recent literature indicates that prenatal poly I:C administration may not induce AMPH hypersensitivity as robustly as earlier research seemed to suggest. For instance, Vorhees et al. (2012) found that exaggerated hyperactivity following an AMPH challenge was only observed in rats born to poly I:C treated-dams (8 mg/kg; GD 15) that had lost or gained the least weight following the treatment. The effect was not observed in offspring born to dams that gained the most weight following poly I:C treatment (Vorhees et al., 2012). As previously discussed, it is possible that our prenatal manipulation simply did not induce the optimal immune response required to induce behavioural deficits in the offspring. Yet another explanation is that maternal infection via prenatal poly I:C treatment does not induce DA hyperactivity. In fact, a recent study found that adult rats born to poly I:C treated-dams (4 mg/kg; GD 15) showed decreased responsiveness to AMPH compared to SAL offspring, a finding that was also dependent on maternal post-treatment weight change (Missault et al., 2014). The authors concluded that the observed

decreased responsiveness reflects alterations in dopaminergic circuits. This finding is consistent with the decreased responsiveness that was observed in our adult poly I:C females. In addition to the reports of decreased sensitivity to AMPH, there have also been reports of decreased locomotor responding in poly I:C offspring following a challenge with the NMDA receptor antagonist, MK-801 (Howland, Cazakoff, & Zhang, 2012; Missault et al., 2014; Vorhees et al., 2012) . MK-801 is frequently reported to induce an increased locomotor response in poly I:C offspring and is thought to do so via downstream effects on DA resulting in increased striatal DA (Meyer, Nyffeler, Yee, et al., 2008; Zuckerman & Weiner, 2005). Thus, although sensitivity to the locomotor-activating effects of MK-801 was not assessed in this experiment, these controversial results are consistent with our findings and demonstrate that poly I:C-induced mesolimbic hyperactivity is not easily replicable.

Effects of poly I:C on PPI of acoustic startle response in juvenile offspring. The acoustic startle response is a cross-species, whole body skeletomuscular response to sudden loud acoustic stimuli which can be modulated by the presentation of a weak acoustic prepulse shortly before the startling stimulus (i.e. PPI of the acoustic startle response). Impairments in PPI of the acoustic startle response are thought to reflect deficits in sensorimotor gating. As previously discussed, the impairments in sensorimotor gating observed in schizophrenia are thought to lead to excessive processing of irrelevant stimuli resulting in cognitive overload and disordered thinking. The ability to demonstrate a deficit in PPI is therefore considered to be a hallmark feature of animal models of schizophrenia-like symptoms. While adult PPI deficits have been well documented, the timing of the onset of PPI impairments has not been examined as

thoroughly. More specifically, researchers have effectively shown that poly I:C treatment at GD 9 in mice and 15 in rats effectively results in impaired PPI of the acoustic startle response in adult offspring (Ozawa et al., 2006; Smith et al., 2007; Wolff & Bilkey, 2010). There is debate, however, about whether such deficits have a purely post-pubertal onset or whether they can also be manifested in juvenile offspring. Thus, our goal was, firstly, to assess whether our prenatal poly I:C treatment would result in impaired PPI in adult offspring, and secondly, whether or not juvenile poly I:C offspring would exhibit impaired PPI.

For previously discussed reasons, we were unable to assess PPI in the adult rat offspring which is especially unfortunate given the conflicting results in the current literature. Furthermore, not knowing whether or not the adult poly I:C rats would have exhibited a deficit in PPI greatly complicates the interpretation of our juvenile results. We found that neither male or female juvenile poly I:C offspring showed deficits in PPI of the acoustic startle response compared to SAL rats. These findings can indicate several things.

On one hand, our results may be consistent with previous reports that prenatal poly I:C administration results in a post-pubertal, but not pre-pubertal emergence of PPI deficit in mice (Meyer, Nyffeler, Yee, et al., 2008; Ozawa et al., 2006; Vuillermot et al., 2010). This maturational delay is indicative of a progression of pathological symptoms from pre-pubertal to adult life, which is consistent with the post-pubertal onset of full-blown psychotic behaviour in schizophrenia (Rapoport et al., 2005). On the other hand, the literature using rats as subjects appears to be more convoluted. While some researchers report no PPI deficits in adult poly I:C- offspring (Missault et al., 2014),

others report that prenatal poly I:C produces PPI deficits of the acoustic startle response in both juvenile and adult rats (Wolff & Bilkey, 2008, 2010). According to this view, it is possible that the observed absence of PPI deficits in our study is indicative of technical faults in our prenatal manipulation. For instance, given the arguably important effect of maternal weight gain in magnitude of deficits, some may argue that our inability to induce PPI deficits in the juvenile offspring indicates that our poly I:C injections did not induce the threshold maternal immune response required to produce behavioural deficits in the offspring. This, however, seems unlikely, as there have been reports that PPI differs from other forms of behaviour in that it does not appear to be associated with maternal weight loss (Wolff & Bilkey, 2010).

Finally, when comparing our results to those of the literature, our small sample size should be considered a serious limitation. For instance, with our male rats, we did not find a statistically significant main effect for prenatal treatment condition and, consistent with this, our effect size was small ($\eta_p^2 = 0.15$). Wolff and Bilkey (2008), however, report an effect for prenatal treatment in juvenile rats that was not statistically significant, but at trend level. Interestingly, the effect size in their study was of the same magnitude as ours ($\eta_p^2 = 0.15$). Even more important is the fact that the statistically significant effect of prenatal treatment on PPI in adult rats reported by Wolff and Bilkey (2008) was also of a similar magnitude to their trend-level juvenile effect and our non-significant effect in juvenile males ($\eta_p^2 = 0.14$). This suggests that an increase in sample size may very well have revealed a statistically significant effect for prenatal poly I:C in our juvenile male rats. Unfortunately, without the adult data, our PPI results remain inconclusive.

Concluding remarks. Our overall results did not support the general hypothesis that prenatal infection would induce behavioural deficits in adult, but not juvenile offspring. As described in detail above, there are many explanations as to why our prenatal manipulation failed to induce behavioural deficits that have been reported in previous research. It is also possible that there is truth to our findings and that poly I:C does not in fact induce behavioural alterations in the offspring of treated dams. Indeed, discussions with many scientists at different neuroscience conferences suggest that our findings are far from being unique. Many researchers, including a past graduate student in our laboratory, have attempted and failed at replicating poly I:C findings. Unfortunately, given scientific journals' biases towards statistically significant findings, these impressive amounts of null findings are filed away. The important take-home message from the work presented in Chapter 1 of this thesis is that the poly I:C model of schizophrenia-like behaviours is far from being as robust as the literature seems to suggest.

Chapter 2: Behavioural and neurochemical consequences of chronic prenatal MK-801 administration in the juvenile and adult rat offspring

Experiments in Chapter 2 were designed to examine whether blockade of NMDA receptor during prenatal development would result in altered behaviour and neurochemistry in adult, but not juvenile offspring. More specifically, we aimed to replicate unpublished findings from our collaborators (Madularu et al., unpublished) indicating that adult offspring born to MK-801-treated dams displayed deficits in object recognition memory and sensitivity to the effects of drug challenges. Furthermore, we predicted that MK-801-treated offspring would show cognitive impairments, attentional

deficits, anhedonia-like symptoms, and altered DA activity. Given the critical role of NMDA receptors in the establishment of normal neural networks in the developing brain, and consistent with the early adulthood onset of symptoms in schizophrenia, we hypothesized that these deficits would have a post-pubertal emergence. Although our findings do not support the conceptualization of prenatal MK-801 as a model of schizophrenia-like behaviours, our results suggest that prenatal NMDA receptor blockade induced changes in neurodevelopment that resulted in clear deficits in adult offspring.

Effects of prenatal MK-801 on body weight during development. In order to confirm the effects of prenatal MK-801 administration on development, we observed the body weight of pups at various time points throughout development. We did not find any differences between SAL and MK male pups at any of the time points. However, we found the MK females weighed significantly less at PND 2 compared to SAL females of the same age. This difference quickly disappeared with no body weight differences observed between SAL and MK rats at PND 7, 14, or 21. These results are inconsistent with unpublished research from our collaborators that found that male MK offspring weighed significantly less than SAL offspring at PND 8. In line with our findings however, Lu et al. (2011) found no significant differences in body weight between rats born to PCP-treated dams and those born to SAL-treated dams. Despite the absence of body weight differences, the prenatal PCP manipulation resulted in impaired NOP, increased immobility in a forced swim task, and attenuated glutamate transmission in the PFC (Lu et al., 2011). These findings suggest that lower pup body weight as a result of prenatal PCP, and possibly MK-801, administration is not necessary to induce behavioural alterations in the adult offspring.

Effects of prenatal MK-801 on novel object preference in juvenile and adult offspring. As previously discussed, recognition memory has been shown to be disrupted in patients with schizophrenia. In addition, researchers have shown an important role for glutamate in object recognition memory whereby NMDA receptor blockade via MK-801 injections induces impairments in the NOP task at both short and long retention delays (Winters & Bussey, 2005). Furthermore, in a previous study, Lu et al. (2010) showed that prenatal exposure to the NMDA receptor antagonist, PCP, resulted in impaired object recognition memory following a 24 h retention delay in adult mice offspring. Thus, we hypothesized that adult but not juvenile rats born to MK-801-treated dams would show impaired NOP. This hypothesis was partially supported in adult male rats where SAL rats showed NOP following a 90 min delay while the MK rats did not. This result suggests that prenatal NMDA receptor blockade induced neurodevelopmental changes that resulted in altered NOP in adult male offspring at this delay.

Consistent with our findings from Chapter 1, we were unable to observe NOP in either the SAL or MK adult males following a 4 h delay. Curiously, this is inconsistent with previous findings of NOP in adult control Sprague-Dawley rats following up to a 24 h delay (Reger et al., 2009). Of course, NOP in this experiment was constrained by the same limitations discussed previously. Briefly, interpretation of NOP results is limited to situations in which animals show NOP above chance level while a lack of NOP may not necessarily reflect impairment in object recognition. Thus, it is likely that other factors, such as the failure to counterbalance retention delays, may explain why the rats failed to show NOP at a 4 h delay. Interestingly, in an unpublished set of experiments, Madularu et al. showed that SAL rats showed significant NOP following a 4 h delay when tested at

PND 60, while these same rats failed to show NOP following the same delay at PND 210. These results are consistent with the suggestion that NOP is age-dependent such that older rats are less likely to discriminate between familiar and novel objects (Scali et al., 1997). However, the rats in our study (approximately 3 months old) and Madularu et al. (7-8 months old) were considerably younger than the 24 months old rats used in Scali et al.'s study. Thus, age-related performance deficits in the NOP cannot reliably explain the lack of NOP at the 4 h delay reported here.

As was the case with our poly I:C experiments, our predictions about NOP in juvenile offspring could not be supported. We found that neither the SAL nor the MK juvenile male offspring showed NOP following 90 sec and 4 h delays. To further complicate interpretation, both groups showed NOP at a 15 min delay. The fact that NOP was observed at the 15 min delay, but not at the 90 sec delay suggests that factors other than impaired object recognition may explain our results. As previously discussed, future studies examining NOP in juvenile rats should use objects previously tested for innate preference in juvenile rats and use a circular arena, as opposed to the square arena used here, to reduce time spent in corners (Heyser & Ferris, 2013).

The pattern of results for juvenile and adult females was complex. Consistent with the male data and the results from our poly I:C experiments, we were unable to show NOP in adult female rats following a 4 h delay. However, following a 90 min delay, MK females showed NOP while the SAL females did not. Because we failed to observe the expected NOP in control rats after this delay, we cannot interpret results from the MK rats. It should be noted that although NOP in female SAL rats was not statistically significant, the magnitude of the effect (d = .63) was comparable to that of

the MK adult males that reached statistical significance at a 15 min delay (d = .57). Considering effect size alone, this pattern of results suggests the prenatal MK-801 treatment did not induce altered NOP in adult female offspring. Juvenile female results, as was the case with male juvenile offspring, were inconclusive.

Effects of prenatal MK-801 on AMPH and MK-801-induced locomotor activity in juvenile and adult rat offspring. As previously discussed, sensitivity to the locomotor activating effects of AMPH has become a hallmark phenomenon for animal models of schizophrenia-like behaviours and is thought to reflect mesolimbic DA hyperactivity which is associated with positive symptoms of schizophrenia. Furthermore, induction of hyperlocomotoion via NMDA receptor antagonists, such as MK-801, is proposed to model the NMDA receptor-mediated hypoglutamatergic pathophysiology of schizophrenia (Javitt & Zukin, 1991). We therefore hypothesized that adult offspring born to MK-801-treated dams would show increased locomotor activity following an AMPH and an MK-801 challenge compared to SAL offspring. These hypotheses were not supported with the female data where we failed to observe any differences between SAL and MK offspring. The male offspring data also did not support our hypothesis about sensitivity to AMPH. Interestingly, we found that while there were no differences between groups during the juvenile period, adult male MK offspring showed reduced sensitivity to the locomotor-activating effects of MK-801.

This result was the exact opposite of what we had predicted – rather than displaying enhanced sensitivity to MK-801, as would be expected of an animal model of schizophrenia-like behaviour, we observed a marked reduction in MK-801-induced locomotor activity in MK offspring compared to control rats. These results are

inconsistent with previous findings showing that prenatal exposure to MK-801 from E15-18 in mice resulted in enhanced PCP-induced hyperlocomotion in adult offspring (Abekawa et al., 2007). The authors propose that prenatal MK-801 administration disrupts GABAergic neurodevelopment in the medial PFC and that this disruption in GABA neurons development is related to the enhancement of the locomotor-activating effect of PCP that they observed. Further research would be necessary to determine whether similar or opposite changes occurred using our protocol. Interestingly however, our AMPH results are somewhat consistent with those of Abekawa et al. (2007) who found no differences between MK and SAL rats in sensitivity to the locomotor-activating effects of psychostimulant drugs (in their case, methamphetamine). The authors make the interesting proposition that psychostimulant-induced hyperactivity observed in other models can serve as a model of DA D2 receptor antagonist-responsive pathology (Abekawa et al., 2007). Thus, perhaps our model that showed no AMPH-induced hyperactivity may model DA D2 receptor antagonist-resistant pathology.

It is possible that there was an age-dependent development of compensatory mechanisms that occurred in our rats to compensate for a loss of GABAergic neurons in the mPFC induced by prenatal MK-801 exposure. Indeed, Madularu et al. (unpublished) reported that PND 60 rats born to MK-801-treated dams showed increased locomotor activity following an MK-801 challenge. Interestingly, in this same experiment, they found no differences between MK and SAL offspring in response to an MK-801 challenge when rats were tested at PND 210. Rats in the present experiment were between PND 150-170 at the time of these tests. Thus, it is possible that we are witnessing age-dependent changes in response to drug challenges, which progressively

develops due to the compensatory mechanisms mentioned above. Importantly, the sensitivity to the locomotor activating effects of PCP in prenatal MK-801 exposed rats was observed in PND 63 rats, (i.e., young adults) (Abekawa et al., 2007). Future studies can attempt to shed light into these results by examining drug sensitivity in MK and SAL offspring at different stages of adulthood and combining these behavioural observations with molecular techniques that would allow testing for changes in GABAergic neurons.

As was previously discussed in the context of our poly I:C experiment, it is important to keep in mind that existing putative neurodevelopmental animal models of schizophrenia-like behaviours do not induce AMPH and MK-801 hypersensitivity as consistently as was once believed. For instance, (Missault et al., 2014) found that prenatal poly I:C resulted in reduced responsiveness to AMPH in adult offspring. Consistent with our effects of prenatal MK-801 administration, several authors have reported decreased sensitivity to MK-801 in offspring born to poly I:C-treated dams (Howland et al., 2012; Missault et al., 2014; Vorhees et al., 2012). Thus, it is unclear whether our results can be interpreted in the context of a schizophrenia model. It is clear, however, that prenatal MK-801 disrupted normal brain development resulting in a post-pubertal emergence of reduced sensitivity to MK-801.

Effects of prenatal MK-801 on working memory in a delayed-non-match to place task in adult male offspring. Working memory, the ability to hold and manipulate information in order to perform various tasks (Baddeley & Della Sala, 1996), has been shown to be impaired in patients with schizophrenia (Green & Nuechterlein, 2004). Lesions of the ventral hippocampus in neonates has been shown to induce deficits in working memory (Lipska et al., 2002). These findings suggest that interference with

normal development in the circuitry of the hippocampus and the PFC induces working memory deficits that are consistent with findings in schizophrenia (Castner, Goldman-Rakic, & Williams, 2004). In addition, brief disruption of NMDA receptors via MK-801 administration in rats on PND 7 through 10 (i.e. equivalent to prenatal second trimester of primate brain development) resulted in impaired spatial working memory (Stefani & Moghaddam, 2005). As working memory has yet to be assessed in a prenatal NMDA receptor blockade model, we tested whether prenatal MK-801 would induce working memory deficits in a T-maze based DNMP task in adult offspring. We found no differences in working memory in SAL and MK rats following 5, 15, and 20 min delays. Thus, our findings suggest that prenatal MK-801 exposure does not induce working memory deficits, as measured with this task.

The absence of deficits in working memory again questions whether prenatal NMDA receptor blockade can be used as an animal model of schizophrenia-like behaviour. An important consideration when examining working memory in the context of schizophrenia is the extent to which the rodent task used to assess working memory taps into the same process that is compromised in schizophrenia (Castner et al., 2004). Tasks that are designed to test working memory in rodents may also involve episodic memory, thereby recruiting the hippocampus, in addition to the PFC, especially when intervals are long (min vs. sec) (Castner et al., 2004; Kesner & Rolls, 2001). Thus, it is possible that given the long delays used in the present experiment, recruitment of the hippocampus may have compensated for any changes in PFC circuitry resulting from our prenatal manipulation.

In an effort to maximize the probability of observing a deficit in working memory, we challenged the rats with AMPH prior to tests on the DNMP task with a 20 min retention delay. The rationale for this procedure was that if prenatal MK-801 had in fact induced changes in mesocorticolimbic DA circuitry, challenging this system with a DA agonist might help reveal a difference in working memory between the SAL and MK rats that is otherwise not apparent. Furthermore, acute injections of high doses of AMPH have been shown to disrupt working memory in a delayed non-match to place task (Shoblock, Maisonneuve, & Glick, 2003). However, no differences were observed between our two treatment conditions. In retrospect, this result is not surprising given the fact that we did not observe differences between SAL and MK rats in sensitivity to the locomotor-activating effects of AMPH. As we did see a between-group difference in sensitivity to MK-801, it would be interesting to test for working memory differences in SAL and MK offspring following the injection of an NMDA receptor antagonist.

Effects of prenatal MK-801 on social interaction in adult male offspring. As previously discussed, there is a particular lack of effective treatment for primary negative symptoms in schizophrenia. The human literature supports a two factor model of negative symptoms in which blunted affect and poverty of speech construct an expressive deficit domain, while anhedonia (discussed next), asociality, and avolition form an avolition domain (Kirkpatrick & Fischer, 2006; Liemburg et al., 2013). Readily measured in animals, anhedonia and social withdrawal are the two most common negative symptoms modelled in animals (Ellenbroek & Cools, 2000). NMDA receptor antagonism in adult rats via PCP, MK-801, or ketamine has been shown to reliably induce social withdrawal (Gururajan, Taylor, & Malone, 2010; Neill et al., 2010; Neill et

al., 2014). To a much lesser extent, social withdrawal has also been examined in neurodevelopmental models with prenatal poly I:C resulting in social withdrawal in adult offspring mice (Smith et al., 2007) and prenatal administration of the influenza virus inducing social deficits (Shi et al., 2003). Social interaction has not, to my knowledge, been assessed in a prenatal NMDA receptor blockade model. We thus hypothesized that rats born to MK-801-treated dams would show signs of asociality in an open-field social interaction task.

Our results did not support this hypothesis, but did nonetheless give rise to an interesting pattern. Although we failed to observe any statistically significant differences, we found that MK rats spent less time sniffing and more time wrestling the conspecific compared to SAL rats. This is, to a certain extent, consistent with previous research showing that subchronic treatment with PCP or prenatal poly I:C administration results in social withdrawal in adult rodents (Gururajan et al., 2010). However, Neill et al. (2014) reports that analysis of 8 separate studies revealed that in every study reduction of "following" behaviour in PCP-treated rats was observed. In the present study, we did not find any differences between MK and SAL rats in following behaviour. Nonetheless, our results appear to point towards a pattern of reduced prosocial behaviour in MK offspring which would merit further investigation.

Unfortunately, the between-subject variability in this task complicated the interpretation of our results. Our procedure was not without limitations. Similar to the commonly used experimental procedure described in Neill et al. (2014), our rats were matched by weight, habituated to the testing environment on days prior to testing, and pairs of rats, unfamiliar with each other, were placed in the arena for 10 min. Unlike this

procedure, however, our rats were individually caged for weeks prior to testing which may have affected social behaviour. Another important feature in the social interaction test that was left out in our procedure is the presence of an inanimate novel object inside the testing arena. This procedure allows the measurement of any differences in interaction of the test animal with an unfamiliar rat as opposed to an unfamiliar object thus allowing the researcher to make sound statements about social preference rather than general novelty preference (Neill et al., 2014). Finally, in addition to measuring sniffing, following, mounting, and wrestling, some researchers also assess for avoidance (actively turning away or freezing when approached by the conspecific rat). In addition to the decreased following behaviour, an increase in avoidance behaviour in PCP-treated rats compared to controls is commonly reported (Neill et al., 2014).

Although the abovementioned changes to our protocol may have led to different results, the open-field social interaction protocol, as described by Neill et al. (2014), also has its limitations. In this procedure, rats are always paired with another rat of the same treatment condition and social behaviour is based on the way in which the pair interacts. The assumption here is that rats of the same treatment condition will behave in similar ways. The between-subject variability in our experiment suggests that this assumption is likely violated and that measures of social behaviour that control for such confounds would perhaps produce more reliable results. One such procedure is the three-chamber test of sociability and preference for social novelty (Moy et al., 2004). In the sociability test, the test rat is given a choice between spending time in the side with an unfamiliar rat (enclosed in a small wire cage allowing for nose contact between bars) versus an empty side. In the test of preference for social novelty, the rat is given the choice between the

first unfamiliar rat and a newly introduced unfamiliar rat (Moy et al., 2004). One may even wish to add a third test which would differentiate social novelty preference from general novelty preference by giving the test rat the choice between an unfamiliar rat and an unfamiliar object.

Effects of prenatal MK-801 on sucrose preference in adult male offspring. A highly appealing feature of the NMDA receptor-blockade approach for schizophrenia models is that unlike AMPH challenge, which is mostly relevant to the positive symptoms, NMDA receptor antagonists also mimic negative symptoms such as anhedonia. PCP administration in adult rats has been shown to decrease interest in pleasurable stimuli such as sucrose (Baird, Turgeon, Wallman, & Hulick, 2008; Turgeon & Hoge, 2003) and intracranial self-stimulation (Spielewoy & Markou, 2003) suggesting a possible anhedonic effect. Our hypothesis that prenatal MK-801 would result in a lack of preference for sucrose water in adult male rats was partially supported.

We found that MK rats did not show preference for sucrose water, suggesting that, contrary to the SAL rats, they were indifferent to the sweetened water. These results suggest that prenatal MK-801 induces anhedonia-like behaviours in adult offspring. This anhedonia-like behaviour in MK offspring was restricted to conditions where rats were not water or food restricted at the time of testing. When rats were tested under deprivation conditions, neither the SAL nor MK offspring showed sucrose preference. These results are consistent with previous work showing that rats with hippocampal lesions and control rats did not show sucrose preference when tested under deprivation conditions (T. S. Brown & Murphy, 1973). When tested under non-deprivation conditions, however, the lesioned rats showed no preference for the sucrose solution

while the control rats did. Furthermore, these findings implicate the hippocampus in the ability to express pleasure (T. S. Brown & Murphy, 1973). Indeed, in the anhedonic chronic mild stress model (Grippo et al., 2005), various hippocampal changes have been observed. These changes include downregulaion of cannabinoid-1 receptors and lower GABA levels (Gronli et al., 2007; Reich, Taylor, & McCarthy, 2009). Thus, it is possible that blocking NMDA receptors during prenatal development resulted in changes in the hippocampus that are associated with a decreased ability to experience pleasure.

The literature on the role of the hippocampus in anhedonia, however, is inconsistent. For instance, Jenkins et al. (2010) found that persistent blockade of NMDA receptors in adult male rats resulted in reduction in the expression of parvalbumin immunoreactive neurons in the CA1 and CA2/3 regions of the hippocampus. However, these hippocampal changes were not associated with absence of sucrose preference when tested with a 1% sucrose solution. The authors conclude that although subchronic PCP may not serve as a valid model for anhedonia-like symptoms, it induces changes in hippocampal subregions that mimic pathology observed in schizophrenia. Given that in the current study differences in sucrose preference were only observed when rats were tested with a 5% sucrose solution, one may predict that the PCP-induced changes in hippocampal structure would be associated with absence of sucrose preference if tested with a higher concentration sucrose solution. Furthermore, in Jenkins et al.'s (2010) study, rats were tested for sucrose preference 1 and 2 weeks after PCP dosing ended while brain pathology was measured 4 weeks after the final drinking session (Jenkins, Harte, & Reynolds, 2010). Thus, one cannot conclude from this study that anhedonia is not affected by pathology in the hippocampus.

Our sucrose preference procedure was not without its limitations. First, rats did not receive pre-exposure to the sucrose solution. Such pre-exposure to the solution would have allowed a period of adaptation. It is possible that our inability to show sucrose preference in the SAL rats under deprivation conditions (the initial test) was the result of novelty aversion.

Effects of prenatal MK-801 on set-shifting in adult male offspring. Cognitive flexibility refers to the ability to shift from one learned strategy to another (i.e. strategy set-shifting) or from one response pattern to another (i.e. reversal learning), based on changes in environmental contingencies (Ragozzino, 2007). Patients with schizophrenia display perseverative deficits on measures related to cognitive flexibility such as attentional set-shifting (Leeson et al., 2009; Pantelis et al., 1999) and reversal learning (Leeson et al., 2009; McKirdy et al., 2009; Murray et al., 2008; Pantelis et al., 1999). Cognitive flexibility has been assessed in several neurodevelopmental animal models of schizophrenia-like behaviours but has yet to be characterized in the prenatal NMDA receptor blockade model. We therefore hypothesized that adult male rats born to MK-801-treated dams would show impaired cognitive flexibility compared to SAL rats in a T-maze set-shifting task.

Our hypotheses were only partially supported, but an interesting pattern of results emerged. We found that MK offspring appeared to be impaired during set-shifting from a visual-cue strategy to an egocentric spatial strategy, or vice versa. This was reflected in the apparent, but not statistically significant, differences between MK and SAL rats in number of trials to reach criterion during the post-shift phase. Such an impairment suggests that prenatal NMDA receptor blockade disrupts normal development of the

circuit that has been shown to mediate strategy set-shifting which includes prefrontal cortical, thalamic, and striatal areas (Block, Dhanji, Thompson-Tardif, & Floresco, 2007; Floresco, Block, & Tse, 2008; Floresco et al., 2009; Ragozzino, 2007; Ragozzino, Detrick, & Kesner, 1999). Importantly, analysis of error types in our experiment suggest that this apparent deficit was maintained not by perseverative errors, but by significantly more regressive errors in MK rats compared to SAL rats. This finding contradicts previous research with various neurodevelopmental animal models of schizophrenia-like behaviours that have found perseveration to be responsible for impaired set-shifting. For instance, neonatal ventral hippocampal lesions increase perseverative errors during strategy set-shifting in a T-maze task (Brady, 2009), prenatal treatment with methylazoxymethanol acetate impairs extra-dimensional set-shifting and reversal learning in an attention-based task (Featherstone, Rizos, Nobrega, Kapur, & Fletcher, 2007), and prenatal poly I:C increases perseverative errors in a set-shifting operant-based task (Zhang, Cazakoff, Thai, & Howland, 2012). Lesion to the medial PFC or thalamus (Block et al., 2007; Floresco et al., 2008; Ragozzino et al., 1999) as well as dopaminergic receptor manipulations in the mPFC (Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Ragozzino, 2002) and NAcc (Haluk & Floresco, 2009) have been shown to result in increased perseverative errors in set-shifting tasks. However, as perseverative errors were not increased in MK offspring, it appears that these areas may have been spared and that our prenatal manipulation resulted in changes in other structures that are not necessary for the initial shift from one strategy to another.

In a sophisticated series of experiments reviewed in Ragozzino (2007), the authors examined whether the inactivation of the prelimbic cortex and the orbitofrontal

cortex would affect separate processes enabling cognitive flexibility. Furthermore, because both these regions project to the dmSTR, the authors also examined the effects of dmSTR inactivation on set-shifting. The authors showed that the prelimbic area appears to support cognitive flexibility in tasks where conditions require a shift in strategies or rules (e.g. strategy set-shifting) while the orbitofrontal cortex supports learning when the general strategy remains the same but a shift in specific choices is required (e.g. reversal learning in discrimination tests). Notably, in contrast to prefrontal cortex subregions, dmSTR inactivation did not increase perseverative errors, but rather led to an increase in regressive errors. This finding suggests that the dmSTR does not facilitate the initial shift away from a previous strategy, but it is critical for the maintenance of a new strategy. Therefore, it is possible that prenatal blockade of NMDA receptors resulted in changes in the dmSTR that would have led to increased regressive errors, while leaving the prelimbic cortex intact. Given our set-shifting results and the findings described in Ragozzino et al. (2007), the dmSTR may be a good place to begin the search for the specific neural adaptations induced by prenatal NMDA receptor blockade. Indeed, researchers have shown that higher levels of NMDA receptor binding in the dmSTR were correlated with poorer set-shifting performance in adult male Long-Evans rats (Nicolle & Baxter, 2003). Chronic prenatal MK-801administration may have resulted in increased glutamate release or upregulation of NMDA receptors in the dmSTR.

In conclusion, prenatal NMDA receptor blockade resulted in impaired performance in a set-shifting task. However, because the impairment was driven by regressive but not perseverative errors, more research is required to determine whether

this deficit reflects the impairment in cognitive flexibility observed in patients with schizophrenia.

Effects of prenatal MK-801 on AMPH-induced DA activity in adult male offspring. Given the tenacity of the DA hypothesis in schizophrenia research, examining DA activity in the brains of our prenatally MK-801 treated rats is highly relevant. Seeing as we failed to find differences between SAL and MK rats in sensitivity to the locomotor-activating effects of AMPH, which is commonly reported with animal models of schizophrenia-like behaviours, we chose to examine DA activity following an AMPH challenge. The literature on DA activity with neurodevelopmental animal models of schizophrenia-like behaviours is inconsistent with researchers reporting both increases and decreases in DA activity depending on the model used, timing of manipulation, postnatal age of the offspring, and brain region examined (Boksa, 2010). We thus hypothesized that we would observe differences between MK and SAL rats in DA activity in the NAcc, dmSTR, dlSTR, and vPFC. Once again, this hypothesis was only partially supported with trends towards decreased DA turnover in the NAcc, dmSTR, and dlSTR.

Although DA activity in the prenatal NMDA receptor blockade model has yet to be characterized, DA activity has been examined in other neurodevelopmental animal models. For instance, a large number of research groups using prenatal LPS administration in the rat report decreased DA levels or innervation in the NAcc or STR which is consistent with our results (Bakos et al., 2004; Ling et al., 2002; Romero et al., 2010; Snyder-Keller & Stark, 2008). On the other hand, other researchers using this same model have reported increased DA levels and tyrosine hydroxylase (TH; rate-

limiting enzyme in DA synthesis) immunoreactivity in the NAcc (Borrell, Vela, Arevalo-Martin, Molina-Holgado, & Guaza, 2002; Romero et al., 2007; Romero et al., 2010). Research with prenatal poly I:C appears to provide more evidence for increased DA activity in the offspring. For instance, researchers have shown increased KCl-induced DA release from striatal slices prepared from rat offspring born to poly I:C-treated dams (Zuckerman et al., 2003) and increased TH immunoreactivity in the NAcc and STR and increased levels of DA in the PFC of adult poly I:C mice (Meyer, Engler, Weber, Schedlowski, & Feldon, 2008; Meyer, Nyffeler, Schwendener, et al., 2008; Meyer, Nyffeler, Yee, et al., 2008; Winter et al., 2009). Others have reported increased DA turnover in the STR of adult mice prenatally treated with poly I:C from GD 12 to 17 (Ozawa et al., 2006).

Due to unfortunate technical complications with our HPLC procedure and the absence of statistically significant results, we cannot confidently state that MK rats do in fact exhibit reduced DA activity. Nonetheless, these results are interesting as they corroborate some of our behavioural observations. More specifically, reduced DA activity following an AMPH challenge may explain why we failed to see differences in AMPH-induced locomotor activity and reduced sensitivity to the locomotor-activating effects of MK-801. Furthermore, reduced DA turnover in the dmSTR, an area shown to be critical for the maintenance of a new strategy (Ragozzino, 2007), may help explain why MK rats made significantly more regressive errors than SAL rats in our set-shifting task.

Concluding remarks. Results from this extensive project do not support the general hypothesis that prenatal MK-801 administration results in schizophrenia-like

behaviour in adult offspring. However, it should be clear to the reader that our prenatal manipulation did in fact induce changes in offspring neurodevelopment that gave rise to behavioural, and possibly neurochemical, alterations in the adult offspring. Although our findings were sometimes in the opposite of our hypotheses and further research will be required to determine the exact nature of these alterations, we feel that the results are interesting in their own right and contribute to the advancement of our understanding of the role of NMDA receptors in development and behaviour.

We hope that future studies will expand on our findings with molecular techniques that would help elucidate the changes observed in our animals. An important future direction would be to examine the potential compensatory mechanisms that emerged as a result of NMDA receptor blockade. One promising direction is suggested by Lu et al. (2011), who identified changes in glutamate levels and expression of glial glutamate transporters in prenatal PCP offspring. Another important avenue would be replicate and expand on the findings of Abekawa et al. (2007) who found that prenatal MK-801 administration disrupted GABAergic neurodevelopment in the mPFC of rat offspring.

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