

**Prenatal PolyI:C induced schizophrenia-like cognitive inflexibilities in the male, but
not female, rat adult offspring**

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ABSTRACT

Executive functions are important cognitive processes critical for survival. Damage to the prefrontal cortex impairs executive functions, such as working memory, decision making and set-shifting. Interestingly, patients diagnosed with different psychiatric disorders are also impaired in executive functions, especially in the set-shift domain, often measured by the Wisconsin Card Sorting Task (WCST). Set-shifting is an essential cognitive process, in that it allows the individual to suppress non-reinforcing strategies and engage in new rewarding strategies. To date, little is known about the etiology of executive dysfunction in psychiatric disorders. However, some epidemiological and serological experiments have shown strong correlations between prenatal infection and the increased risk to develop psychiatric disorders in the adult offspring. One study found that schizophrenic patients pre-exposed to a prenatal infection perseverated more during the WCST, than non-pre-exposed patients. Despite these findings, there are still numerous limitations (e.g., ethical concerns) when conducting these studies. Thus, animal models are important and can further elucidate the etiology of executive dysfunctions in psychiatric disorders. Prenatal infection animal models have consistently shown that inflammation during gestation in rodents induces behavioural, anatomical and cognitive changes in the adult offspring similar to psychiatric patients. However, no studies have investigated the effects of prenatal infection on set-shifting in the adult offspring. Therefore, the present thesis examined whether prenatal treatment with PolyI:C (a viral mimetic) during middle/late gestation of the rat would induce cognitive inflexibilities (i.e., set-shifting and reversal learning in an operant based task analogous to the WCST) in the adult male and female offspring. The results showed PolyI:C male offspring perseverated during the set-shift but had fewer regressive errors during the reversal learning day. PolyI:C treated female offspring were

not impaired during any of the test days; however, females were slower to respond to the lever and required more training when compared the male rats. Taken together, these results give support for prenatal infection in inducing cognitive inflexibility, by potentially altering the PFC in the adult offspring.

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DEDICATION

I dedicate this to my family, especially my mom, and my friends for their continuous encouragement, support, guidance, and mentorship in helping me throughout my academic career and more. Words cannot begin to describe my appreciation and gratitude for all of you.

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LIST OF ABBREVIATIONS

5-HT	Serotonin
ABN/LG	Arch-back Nursing/Licking and Grooming
ARL	Average response latency
ADHD	Attention-deficit/hyperactivity disorder
BLA	Basolateral Amygdala
CANTAB	Cambridge Neuropsychological Test Automated Battery
D ₂	Dopamine Receptor Subtype 2
D ₄	Dopamine Receptor Subtype 4
DA	Dopamine
EDS	Extradimensional Shift
FR1	Fixed-ratio 1
GABA	Gama-aminobutyric
GD	Gestational Day
GSK3	Glycogen Synthase Kinase 3
IDS	Intradimensional Shift
IGT	Iowa Gambling Task
IL	Interleukin
LI	Latent Inhibition
LPS	Lipopolysaccharides
mPFC	Medial Prefrontal Cortex
NAc	Nucleus Accumbens
NMDA	N-methyl-D-aspartate
OCD	Obsessive-compulsive disorder

OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex
PND	Postnatal day
PolyI:C	Polyriboinosinic:polyribocytidilic Acid
PPI	Prepulse Inhibition
TTC	Trials to Criterion
WCST	Wisconsin Card Sorting Task
WISC-IV	Wechsler Intelligence Scale for Children

INTRODUCTION

Executive Function: Frontal Lobe Patients & Animal Models

Executive functions are higher order cognitive processes critical for animals to survive and adapt to their immediate environment. In a broad stroke, executive functions are reflected in the ability to plan out a step-by-step approach to solve problems or reach goals. Numerous cognitive skill sets constitute executive functions. These include attention, memory, working memory, planning, temporal integration, decision making, monitoring and inhibitory control (Fuster, 2008). These subtypes of executive function have been well described by Fuster (2008). Briefly, attention refers to the ability to concentrate on a currently relevant entity, while ignoring stimuli that are irrelevant. There are 5 subtypes of attention, which is comprised of alertness (i.e., being aware of one's environment), set (i.e., the ability to engage relevant strategies and disengage irrelevant strategies), spatial attention (i.e., the awareness of spatial orientation of self and objects), sustained attention (i.e., the ability to continuously attend to stimuli that are currently relevant), and lastly, interference control (i.e., being able to filter out irrelevant stimuli from the environment). Memory is the ability to form and remember a representation of some entity or situation from the past, present, or future. Working memory is the ability to retain information that is currently relevant and would be subsequently used to solve a problem. Planning refers to the capability of integrating relevant strategic steps in executing and reaching a goal. Temporal integration refers to the ability to bring together ideas and memories from various time points in one's history and experience to reach a goal. Decision making is the ability to use cues (e.g., from the environment or internal cues) or strategies in deciphering information and select between outputs that would yield the most gain and/or least negative outcomes. Monitoring is the act of being able to regulate one's output; hence, allowing for

modification in thinking or behaviour when necessary. The last key component of executive function is inhibitory control. This is the ability to suppress irrational or impulsive thoughts and behaviours that would yield negative outcomes or impedes one's ability in reaching a goal. In a broader sense, inhibitory control also encompasses the capacity to disregard irrelevant stimuli in the environment by only focusing on relevant stimuli (Fuster, 2008).

These components of executive function have been studied extensively and have been consistently shown to be mediated by the prefrontal cortex (PFC) and its subcortical circuitries (Fuster, 2008; Pantelis et al., 2009; St. Onge & Floresco, 2009). The prefrontal cortex is comprised of many sub-structures (St. Onge & Floresco, 2009). The dorsal lateral PFC has been frequently studied and is strongly implemented in mediating set-shifting discriminations (Floresco et al., 2009). Other sub-regions, such as the orbital frontal cortex and insular cortex, have also been associated with complex decision processes (St. Onge & Floresco, 2009). In humans for example, prefrontal damage resulted in the inability to disregard irrelevant stimuli in the environment (Pantelis et al., 2009). Consequently, patients with prefrontal damage cannot sufficiently concentrate on specified tasks when exposed to irrelevant stimuli that compete for attention (St. Onge & Floresco, 2009). In addition, these patients are impaired on tasks that tap into working memory (Fuster, 2008). Specifically, these working memory impairments are more prominent as the delay between the training and test phase increased (Levy & Goldman-Rakic, 2000). Damage to the Brodmann's area 8, which is a sub-region in the PFC, impairs spatial attention, whereby these patients do not attend to the critical components of an image in a logical manner when trying to understand what the image represents (Fuster, 2008; Levy & Goldman-Rakic, 2000). As well, patients with lateral prefrontal cortical lesions exhibited an inability to

attend to environmental cues that may be critical in displaying appropriate behaviour outputs (Fuster, 2008).

During the Wisconsin Card Sorting Task (WCST), patients with frontal lesions display difficulty in set-shifting, which required the suppression of the old correct strategy and utilization of the new correct strategy (Pantelis et al., 1999). This task required the patient to initially learn to sort a deck of cards using only one dimension type (i.e., the shape, colour or number of shapes on the cards). When the current strategy is no longer correct (e.g., sorting by shape), indicated by the experimenter, the patient is then to suppress the use of the old strategy and switch to the new correct dimension (e.g., either using colour or number of shapes on cards). WCST is an important measure, given that it is a reliable measure of cognitive flexibility and is frequently used by researchers examining this type of cognition (Brown et al., 2009; Cannon et al., 1994; Fuster, 2008; Longenecker et al., 2010; Pantelis et al., 2009; Razani et al., 2010; Seidman et al., 1997). Cognitive flexibility is an essential ability, as deficits in this domain significantly affect one's ability to live independently (Harvey et al., 1998; Razani et al., 2010). For example, cognitive inflexibility disrupts the ability to complete simple tasks, such as grocery shopping (Razani et al., 2010). In addition, majority of patients with psychiatric disorders display cognitive inflexibilities (Hartman et al., 2003; Hasselbalch et al., 2010; Li, 2003; McKirdy et al., 2008; Razani et al., 2010); therefore, it is crucial to examine the potential factors that may induce this deficit.

Patients with prefrontal cortical damage perseverated during the task by continuously using the old, currently incorrect strategy. In addition, these patients had great difficulty in completing the Tower of London test. This particular task required the patient to actively plan out the steps to move wooden rings from one pole to another, in order to match the wooden rings

display that was set by the experimenter (Fuster, 2008). Thus, it seems as though attention shifting, strategic planning and decision making are significantly impaired in individuals with prefrontal cortex lesions. Other frontal sub-regions, such as the orbital frontal cortex (OFC) are implicated in regulating inhibition, memories and decision making that is emotionally linked. Damage to this area induces false memories and lack of inhibitory control (Fuster, 2008). For example, patients with ventromedial lesions of the OFC exhibit poor performance on the Iowa Gambling task (IGT; St. Onge & Floresco, 2009). These patients will continuously select the deck of cards that was considered ‘disadvantageous,’ whereby it gave out a higher reward but also had a higher risk in losing one’s money compared to the other (safe) deck of cards (Fuster, 2008). In addition, OFC mediates reversal learning (Hampshire & Owen, 2006). Functional magnetic resonance imaging demonstrated that during the intradimensional shift, the lateral OFC displays the most activity (Hampshire & Owen, 2006). These data sets clearly show the significance of the PFC in mediating executive functions and that damage to this region results in dysfunctions.

Executive Function in Psychiatric Disorders

Executive dysfunction is not limited to patients with prefrontal cortical damage or lesions. Numerous studies have shown patients with psychiatric disorders exhibit executive dysfunction. In brief, patients with obsessive-compulsive disorder (OCD) were impaired during a delayed alternation learning task, whereby these patients were required to select the 1 box, out of 3 boxes, that contains a coin (Moritz et al., 2009). OCD patients made more perseverative errors during the WCST and made less correct responses during a 20s delay spatial working memory test (Trivedi et al., 2008). Children diagnosed with attention-deficit/hyperactivity disorder (ADHD) are impaired on working memory tasks (O’Brien et al., 2010). While

Alzheimer's patients have profound memory impairments, they are also significantly impaired in visual-spatial tasks and WCST compared to normal controls (Razani et al., 2010). As well, patients with depression are impaired on set-shifting when compared to normal controls (Hasselbalch et al., 2010). With these studies, it is evident that executive dysfunction occurs in many different psychiatric disorders. However, schizophrenic patients are typically described as most impaired during the WCST (Hartman et al., 2003; Li, 2004; Mckirdy et al., 2008; Pantelis et al., 2009; Weiler et al., 2009).

Schizophrenia is a debilitating psychiatric disorder which encompasses not only cognitive but also positive and negative symptoms (Meyer & Feldon, 2010). Positive symptoms refer to auditory hallucinations, psychosis, and delusions (Meyer & Feldon, 2010). Negative symptoms refer to anhedonia, social withdrawal, and blunted affect (Meyer & Feldon, 2010). Roughly 1% of the population is affected by this disorder and there is no treatment that effectively alleviates all of the symptoms (Meyer & Feldon, 2010). Current antipsychotics are known to ameliorate positive symptoms and some negative symptoms, but rarely show an influence on cognitive symptoms (Rodefer et al., 2008). However, studies have shown that the cognitive deficits have the most detrimental effect, given that these deficits have an inverse correlation with social functioning and prognosis for the patient (Harvey et al., 1998, 1999). Set-shifting deficits are frequently seen in schizophrenic patients during WCST (Cannon et al., 1994). A meta-analysis found that schizophrenic patients are significantly impaired on the WCST, whereby they made more errors than normal controls; however, those errors are not only attributed to perseveration (Li, 2003). Hartman and colleagues (2003) found that as the working memory demands increase, schizophrenic patients made more errors in general, when compared to normal controls. Schizophrenic patients also display difficulty in reward based learning, such as reversal learning

or intradimensional shifts (Weiler et al., 2009). Schizophrenic patients made more errors during both the extradimensional and intradimensional shifts during the Cambridge Neuropsychological Test Automated Battery (CANTAB) Intradimensional/Extradimensional (IDED) task. These patients are also impaired in working memory, spatial working memory, sensorimotor gating, latent inhibition, long term verbal memory and attention (Meyer & Feldon, 2010; Pantelis et al., 2009; Zanello et al., 2009).

Sex Differences in Cognitive Flexibilities

Sex differences exist for certain types of cognitive processes, especially those pertaining to executive function (Seidman et al., 1997). Men are predominantly better at visual-spatial tasks than women, such as mental rotation tasks, and this difference exists despite the increase in complexity of the task (Titze et al., 2008). Sex differences in psychiatric patients also exist, whereby female schizophrenic patients exceed male patients in the verbal memory, processing speed and visual memory domains (Longenecker et al., 2010). Male schizophrenic patients are better in working memory domains and visual-spatial tasks (Longenecker et al., 2010).

Interestingly, male patients perform worse than female patients during the WCST by making significantly more perseverative errors than female patients as they are not able to suppress the use of the old, now currently incorrect strategy to sort the cards (Seidman et al., 1997).

Nevertheless, patients with schizophrenia, as a group, perform worse in those cognitive tasks when compared to normal controls (Seidman et al., 1997). Therefore, given that executive dysfunctions are present in a variety of different psychiatric disorders and have differential effects on the sexes, it is important to study the factors that may contribute to executive dysfunctions. One factor that has been strongly associated with executive dysfunction and psychiatric disorders is prenatal infection. Numerous studies have generated correlative data

linking prenatal infection with executive dysfunction and psychiatric disorders (Brown et al., 2009; Brown & Derkits, 2010).

Prenatal Infection, Development and Executive Dysfunction

Recent studies have shown that adverse events during prenatal or perinatal time points could increase the risk of psychiatric disorders in the adult offspring. Specifically, epidemiological studies show a correlation between prenatal infection and the development of psychiatric disorders, such as schizophrenia, in the adult offspring (Brown & Derkits, 2010; Patterson, 2007). For example, individuals born during the peak of the A2 influenza epidemic in England and Wales in 1957, showed an 88% increase in developing schizophrenia (Brown & Derkits, 2010). A similar increase of schizophrenia in the adult offspring was seen during the Poliovirus outbreak in Finland, from 1959-1969 (Brown & Derkits, 2010). In addition, serological studies have shown that exposure to influenza during early or mid-gestation increased the development of schizophrenia by 3 times (Brown, 2006). Specifically, maternal serum was taken to measure the existence of influenza antibodies. Offspring born to these mothers were then followed up during adulthood, whereby interviews were conducted to determine the development of schizophrenia. In this longitudinal study, exposed and non-exposed adult offspring were compared and results showed that exposure to the influenza virus during the first trimester increased the development of schizophrenia by 7 times (Brown, 2006). Other infectious agents, such as rubella, toxoplasmosis and herpes simplex virus type 2, also increased the development of schizophrenia in the adult offspring when exposed during gestation (Brown, 2006; Brown et al., 2005).

Furthermore, Brown and colleagues (2009) found that not only did prenatal infection increase the risk of developing schizophrenia but it also plays a significant role in executive

dysfunction. This group of researchers took serological medical records of pregnant mothers, whom were initially recruited during the 1960's. Only a subset of samples were taken and used for this study and levels of antibodies for influenza and toxoplasmosis were analyzed directly. Offspring that were either exposed or not exposed to an infection prenatally, but developed schizophrenia in adulthood, were examined. A number of cognitive tests were administered; however, of particular interest are the Trail Making Test B and WCST. The Trail Making Test B requires the patient to connect randomly distributed circles that contain either a letter or number. The objective is to connect the circles from number to letter, in a sequential manner (e.g., 1 to A to 2 to B, etc), as quickly and accurately as possible. Schizophrenic patients performed worse than normal controls throughout these two tasks. These attention flexibility impairments are consistent with other studies (Cannon et al., 1994). Interestingly, patients exposed to a prenatal infection performed worse than schizophrenic patients not prenatally exposed to an infection. Exposed schizophrenic patients perseverated more during the set-shifting phase of the WCST. From this, it is evident that prenatal infection may alter fetal prefrontal cortical development; hence, inducing executive dysfunction in adulthood. Further elucidating this developmental disruption hypothesis in affecting executive function is important, given that little is known about how prenatal infection may induce the development of psychiatric disorders and executive dysfunctions.

More controlled studies are needed to clarify these correlations, in that few studies have investigated the direct implications of prenatal infection on executive dysfunction in adulthood and the development of psychiatric disorders. The majority of studies, including the above mentioned ones link prenatal infection with psychiatric disorders and executive dysfunction. These researchers measured antibodies for some infections from maternal serums and correlated

it to the development of psychiatric disorders in the adult offspring. However, numerous confounds still exist. For example, environmental differences in development and the control of other diseases or family history were rarely accounted for in those studies. Due to methodological and ethical limitations in clinical settings, the use of animal models is essential. Better control of confounds and variables will allow us to further delineate the role of prenatal infection in increasing the risk of developing psychiatric disorders.

Animal Models of Prenatal Infection

Numerous studies have looked at the effects of prenatal infection on cognitive, behavioural and neurochemical measures in the adult offspring of rodents (Bitanirwe et al., 2010a; Hao, et al., 2010; Meyer et al., 2008a, c, d; Ozawa et al., 2006; Shi et al., 2003; Zuckerman & Weiner, 2005). Different pathogens have been used successfully to induce an immune response in pregnant rodents. For example, prenatal exposure to lipopolysaccharides (LPS) induced deficits in the adult offspring during the Morris Water Maze task (Hao et al., 2010). During this task, rats were trained to find a hidden platform, located in 1 of 4 quadrants, in a circular pool that was filled with opaque water. Rats were guided towards the hidden platform if they failed to find the platform within the first 120s of each training trial. On the test day, time spent in the quadrant where the hidden platform was initially located was measured. Rats that were prenatally exposed to LPS took significantly more time and longer paths to reach the hidden platform. In addition, these deficits were correlated with a significant increase in hippocampal neuronal loss (Hao et al., 2010). Polyriboinosinic:polyribocytidilic acid (PolyI:C) has also been frequently used in these animal models (Bitanirwe et al., 2010a, b; Meyer et al., 2008b; Ozawa et al., 2006; Shi et al., 2003; Zuckerman & Weiner, 2005). PolyI:C is a viral mimetic, double stranded RNA compound. It acts on Toll-like receptor 3 receptors that are

present in the brain (Meyer & Feldon, 2010). PolyI:C, like LPS, induces the release of pro-inflammatory cytokines, such as interleukin (IL) 1-beta, IL-6 and tumor necrosis factor alpha (Meyer & Feldon, 2010). The imbalance of cytokines in the fetal environment has been argued to play a pivotal role in inducing cognitive and behavioural changes in the adult offspring (Meyer et al., 2008c). PolyI:C injected during early/middle gestation of the mice significantly reduced the expression of reelin and parvalbumin expressing prefrontal neurons in the adult offspring (Meyer et al., 2008c). Reelin is important in migration and lamination of neurons and parvalbumin is an important marker for GABAergic interneurons. Both are critical components in the developing brain and play a key role in cognitive functions. As well, prenatal injection of PolyI:C induced deficits in prepulse inhibition of the acoustic startle response in the mice adult offspring (Shi et al., 2003). The PPI test examines the percent PPI. PPI represents the ability to reduce one's startle response when a weaker tone is introduced before a loud tone. A low percentage of PPI reflects a deficit, whereby the weaker tone before the loud tone does not affect the motor response to the subsequent loud tone. Therefore, this induces a higher startle response in the impaired rat. This is a well-known deficit, frequently seen in schizophrenic patients (Meyer & Feldon, 2010). Exploratory behavior was significantly decreased in prenatally treated adult mice offspring during the open-field and novel-object tests (Shi et al., 2003). In addition, acute administration of ketamine (i.e., a N-methyl-D-aspartate (NMDA) antagonist) increased motor response in the mice adult offspring (Shi et al., 2003). This increased sensitivity to the drug is representative of positive symptoms exhibited by schizophrenic patients (Meyer & Feldon, 2010). The impairments and alterations exhibited by the rodent adult offspring, which were induced by prenatal infection, are similar to the disabilities seen in psychiatric patients. These findings give credibility to the notion that prenatal infection does in fact play a role in

potentially increasing the susceptibility of the adult offspring to develop a psychiatric disorder. However, does prenatal infection induce set shifting impairments, given that some psychiatric patients with different disorders are impaired on this subtype of executive function (as mentioned previously)?

Animal Analogues of the WCST

Numerous cognitive and behavioural tasks analogous to human tasks have been used frequently in the animal literature; however, there are only 3 tasks that are analogous to the WCST (a more detailed comparison can be viewed in Table 1; Birrell & Brown, 2000; Floresco et al., 2008, 2009; Stefani & Moghaddam, 2005).

The first animal task analogous to the WCST is the digging bowl task. It requires the rat to switch from one strategy to another (Birrell & Brown, 2000). During the task, rats are trained to dig for a food reward in one of two bowls that differ only in one dimension (i.e., scent, digging medium or texture of bowls). Rats initially learn to focus on one dimension (e.g., vanilla scent) to receive a reward. Then a shift, either an intradimensional (IDS; e.g., from vanilla to rose scent) or extradimensional (set) shift (EDS; e.g., from vanilla scent to bowl texture or digging medium), is made. Rats must then learn to ignore the old dimension and respond on the new dimension to receive the food reward. Reversal phases are also present during this model; however, the IDS phase of this test is more comparable with other reversal learning tasks (e.g., operant chamber and T-maze). All testing phases (e.g., EDS & IDS) are examined in one day.

The second task is the T-maze (Ghods-Sharifi et al., 2008). The T-maze is a cross maze that always has one arm blocked off to ensure a T configuration. During this task, rats always start in a different arm and are initially trained to enter one arm, using either a response (e.g., always turn left or right) or a cue (e.g., always turn into the arm with the floor cue) strategy to

receive a food reward. The set-shift component for this requires the rats to suppress the old, currently non-rewarding strategy and acquire the new rewarding strategy by switching between different dimensions. The reversal learning component is similar to the intradimensional shift of the digging bowl task.

The last task analogous to the WCST is the operant chamber based task (Floresco et al., 2008, 2009; Zhang et al., 2011). Rats are initially required to learn that one press of the lever dispenses one food reward. After basic lever training, rats are to learn three new rules in a sequential manner (i.e., visual-cue, set-shift and reversal days). During visual-cue discrimination, rats must press the lever with an illuminated stimulus light above it, to receive a food reward. Upon reaching criterion, rats are then trained to ignore the illuminated stimulus light and only focus on one lever (i.e., either the left or right) to receive a food reward. This test day is the set-shifting day. On the last day of testing, rats are required to press the lever that is the opposite from the set-shift day (e.g., if correct lever on the previous day is the right side, then now rat must focus on the left side). This is the reversal learning day. This task is more efficient than the digging bowl or T-maze because more rats can be tested at once. Furthermore, this automated system more accurately translates the data by reducing human error, which can occur more frequently during the other two tasks, given that experimenters are recording the data while testing animals. As well, the operant chambers monitor a wider array of behavioural responses throughout the task, when compared to the digging bowl and the T-maze tasks.

Table 1: Comparison of the Three Animal Set-shifting Tasks

Task	Dimensions	Advantages	Disadvantages	References
Digging Bowl	<ul style="list-style-type: none"> • Odor • Digging medium • Bowl texture 	<ul style="list-style-type: none"> • Dimensions are more relevant to rodents • More testing parameters (e.g., shifts) • Analogous to the WCST 	<ul style="list-style-type: none"> • Increased human error in encoding data • Increased human influence in rodent behaviours • Limited number of behaviour responses measured simultaneously • Habituation to shifts between dimensions 	Birrell & Brown, (2000)
T-Maze	<ul style="list-style-type: none"> • Turn response • Floor visual-cue 	<ul style="list-style-type: none"> • Dimensions used are constant throughout task • More sensitive to response conflict and error subtypes • Analogous to the WCST 	<ul style="list-style-type: none"> • Increased human error in encoding data • Increased human influence in rodent behaviours • Limited number of variables measured simultaneously 	Floresco et al. (2006a)
Operant Chamber	<ul style="list-style-type: none"> • Stimulus Light • Lever response 	<ul style="list-style-type: none"> • Automated (less human influence on rodent behaviour) • Measures more behavioural responses, simultaneously • Sensitive to error subtypes 	<ul style="list-style-type: none"> • Lever responses are unnatural for rodents 	Floresco et al. (2008)

Animal Tasks and Prefrontal Cortical Functions

The impairments exhibited by patients with prefrontal lesions have been replicated in animal studies using tasks that are analogous to the WCST. NMDA antagonist to the mPFC of the rat induces an increase in perseverative errors during a digging bowl task (Stefani & Moghaddam, 2005). In addition, inactivation of the medial PFC (mPFC) of the rat impairs set-shifting during an operant-chamber based task (Floresco et al., 2008). Rats with mPFC inactivation perseverate significantly, whereby these animals are not able to stop following the illuminated stimulus lights above the levers. The perseveration is clearly reminiscent of the deficits seen in patients with frontal lobe damage, during the WCST (Fuster, 2008). Orbital frontal inactivation in the rat impairs reversal learning, but not the set-shift component of the T-maze based task (Ghods-Sharifi et al., 2008). The impairments induced by OFC inactivation in rats are congruent with disabilities seen in patients with OFC damage (Fuster, 2008).

Other animal models, with different manipulations and tasks, have also shown impairments of different executive dysfunctions. Rats that received infusions of gamma-aminobutyric (GABA) agonists to the prelimbic area (i.e., analogous to the human dorsal lateral prefrontal cortex) increased the selection of the risky lever during a risk-based decision making task (St. Onge & Floresco, 2010). This task resembles the IGT, in that rats were required to pick between two levers. One lever would give a constant small reward, the other lever would give a larger reward but is more risky, in that the probability of receiving the large reward decreased overtime. Bilateral inactivation of the basolateral amygdala (BLA) or unilateral activation of the BLA with contralateral inactivation of the anterior cingulate cortex (ACC) resulted in a decrease in motivation during an effort-based decision making task (Floresco & Ghods-Sharifi, 2006). Here, the inactivation of the BLA and ACC reduced the rat's motivation

to jump across a 30 cm barrier to receive a large reward (i.e., 4 reward pellets) and instead picked the arm with no barrier to receive a small reward (i.e., 2 reward pellets) on the T-maze. This lack of motivation was not the result of motor or spatial impairments, given that there was no difference between the treatment and saline groups in picking the large reward arm when an additional barrier was added to the small reward arm (Floresco & Ghods-Sharifi, 2006). As well, sub-chronic treatment with ketamine, an NMDA antagonist, impaired the rat's performance during a delayed win-shift task by increasing the number of trials to reach criterion (Enomoto & Floresco, 2010). In brief, the delayed win-shift task uses an 8 arm radial maze. During training, 4 out of the 8 arms are blocked off and 4 are baited with one reward pellet. A delay of 5- 30 min where implemented between the training and test phases. After the delay, test phase began and rats were required to remember and enter the arms that were previously blocked to receive one food reward. The radial arm maze task taps into working memory and it could be argued that the NMDA receptors in the PFC play a significant role in executive function.

The impairments observed in the rats exposed to PFC manipulations are generalizable to humans in a clinical setting. Credibility can be given to these animal models, in that various frontal cortex manipulations induced similar, if not identical, impairments which were exhibited by patients with frontal lobe dysfunctions (Fuster, 2008). Therefore, it is possible to use animal models to determine precisely how dysfunctions of the prefrontal cortical region cause cognitive impairments, psychiatric disorders, disease and deficits in attention processing in humans. Due to this, much research has been conducted using animal models to examine the role of prenatal infection in potentially increasing the risk of developing psychiatric disorders in adult offspring.

Prenatal and Neonatal Treatment on Cognitive Flexibilities

A number of prenatal treatment studies have looked at a variety of cognitive and behavioural tasks; however, only a limited number of studies have examined complex executive function, such as set-shifting and reversal learning. Meyer et al. (2006a), found that prenatal treatment with 5mg/kg of PolyI:C on gestational day 15 induced reversal learning impairments in Morris water T-maze. Adult mice offspring were trained to swim into either the left or right arm to find the hidden platform. There were 6 trials per day. Mice were allowed 10 min to find the platform and 10 s to sit on the platform, once it was found. Training continued until a criterion of 11 correct arm entrances was chosen within 2 days. Then the platform was moved to the opposite arm that the mice were initially trained to enter. Again, criterion was the same as training days. PolyI:C treated mice offspring took more trials to reach criterion during reversal learning day, when compared to saline treated mice offspring (Meyer et al., 2006a). In contrast, Zuckerman and Weiner (2005) found a facilitation of reversal learning in the prenatally PolyI:C treated offspring during the same water T-maze task. Those offspring took significantly fewer trials to reach criterion when compared to the saline treated offspring (Zuckerman & Weiner, 2005).

Deficits in set-shifting were found in a neonatal developmental paradigm relevant to schizophrenia (Broberg et al., 2008; Brady, 2009). In Brady's (2009) study, male pups underwent a ventral hippocampal lesion between postnatal days 6 to 8. On postnatal day 56, these rats were trained on a T-maze. Male rats that received the lesion were significantly impaired on this task. They took more trials to reach criterion and this was due to a continuous perseveration of using the old but now non-reinforcing strategy. This is consistent with both frontal lobe and schizophrenic patients, whereby they perseverated on the WCST (Fuster, 2008).

Furthermore, Broberg and colleagues (2008) found that treatment with phencyclidine (PCP) on postnatal day 7, 9 and 11 induced deficits in both the male and female adult rats during the extradimensional shift of the digging bowl task. Reversal learning was not affected. Therefore, it seems a number of developmental manipulations, including prenatal infection; induce alterations of set-shifting and reversal learning. However, the consequences of prenatal infection for set-shifting have not been assessed directly. In addition, the conflicting results regarding the effects of prenatal infection on reversal learning must be clarified. The elucidation of the effect of prenatal infection on both set-shifting and reversal learning are important, given that both processes tap into different sub-regions of the PFC.

Rationale and Hypotheses

Executive functions are an important set of cognitive skills that ensure an animal's survival. The prefrontal cortex plays a significant role in mediating these cognitive processes and damage to this area leads to detrimental effects (Fuster, 2008). Patients with number of psychiatric disorders exhibit symptoms of executive dysfunction, especially the ability to set-shift between dimensions during the WCST (Fuster, 2008). The specific factors that cause executive dysfunction in psychiatric disorders still need to be elucidated. Some studies have correlated infection during gestation with the risk of developing psychiatric disorders in the adult offspring (Brown & Derkits, 2010; Meyer & Feldon, 2010). In addition, these correlations have been further examined in animal models, whereby prenatal infection or maternal immune response does induce behavioural, neuroanatomical and cognitive changes in the adult offspring, similar to symptoms exhibited by psychiatric patients (Meyer & Feldon, 2010). However, only a small number of studies have examined the effects of prenatal treatment on cognitive flexibility, in the adult offspring, utilizing animal models (Bitanirwe et al., 2010a; Brady, 2009; Meyer et

al., 2006a; Zuckerman & Weiner, 2005). Therefore, the present experiment investigated whether prenatal treatment with PolyI:C would alter set-shifting and reversal learning in the rat adult offspring. PolyI:C was administered on gestation day 15, which is identical to the Zuckerman & Weiner (2005) study. Gestational day 15 is considered analogous to the human second trimester (Meyer et al., 2009). PolyI:C was chosen because of its effectiveness in inducing similar behavioural, neuroanatomical, and cognitive deficits as schizophrenia (Meyer & Feldon, 2010). More comprehensive details on the strengths and weaknesses of the PolyI:C model in resembling schizophrenia can be seen in Table 2. I tested both female and male adult offspring in an operant chamber based set-shifting paradigm because there are a limited number of studies that examined the sex differences in executive function after prenatal infection.

I hypothesized that PolyI:C would induce an immune response in the dams sufficient to alter normal neurological and developmental processes. These alterations will cause deficits in both male and female adult offspring; however, it is predicted that male offspring will be more impaired during the performance and execution of the task. Performance during set-shifting and reversal learning days will differ between the treatment and control groups, given that studies have shown that different frontal cortical regions mediate these cognitive processes (Floresco et al., 2008; Fuster, 2008; Ghods-Sharifi et al., 2008).

Table 2: Comparison of Prenatal PolyI:C Treatment and Schizophrenia Symptoms

Prenatal PolyI:C Treatment	Schizophrenia Symptoms
<i>Morphological Changes</i>	
<ul style="list-style-type: none"> • Decrease in neurogenesis, reelin, parvalbumin, and NMDA receptors • Increased pyramidal cells, GABA_A and Dopamine receptors 	<ul style="list-style-type: none"> • Reduced glutamate signaling, decreased reelin and parvalbumin • Increased dopamine release in subcortical regions
<i>Behavioural Deficits</i>	
<ul style="list-style-type: none"> • Decreased social interaction, exploration • Hyperlocomotor activity due to increased sensitivity to dopamine agonist and NMDA receptor antagonist 	<ul style="list-style-type: none"> • Reduced social interaction and increased stereotypical behaviour • Increased sensitivity to dopamine stimulating drugs and NMDA antagonists
<i>Cognitive Deficits</i>	
<ul style="list-style-type: none"> • Decreased prepulse inhibition and latent inhibition • Impaired working memory and spatial abilities • Altered reversal learning 	<ul style="list-style-type: none"> • Impaired prepulse and latent inhibition • Impaired working memory, set-shifting, reversal learning
<i>Disadvantages</i>	
<ul style="list-style-type: none"> • Not all infections in humans lead to the development of psychiatric disorders. (Brown & Derkits, 2010) • Prenatal development in rodents is only comparable to the first two trimesters of human pregnancy (no third trimester in rodents) • Not all symptoms fully replicate symptoms exhibited by schizophrenia patients. • Prenatal infection is not the only developmental variable associated with the development of schizophrenia. 	

METHODS

Subjects

Timed pregnant Long-Evans dams (gestational day (GD) 7; Charles River Laboratories, Quebec, Canada) were singly housed in transparent plastic cages in a temperature controlled (21 °C) colony room on a 12/12 hr light/dark cycle with food (Purina Rat Chow) and water available *ad libitum*. All experiments were conducted during the light phase of a 12:12 h light/dark cycle (lights on at 0700h). All experiments were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Care and Use Program, as well the Animal Research Ethics Board (AREB).

Prenatal treatment

On GD 15, dams (n=20) were individually transported to a room where weight and rectal temperature (Homeothermic Blanket System, Harvard Instruments, MA) were measured. Dams were then anesthetized with isoflurane (5% induction and 2.5% maintenance) and injected intravenously with a single dose of either saline or PolyI:C (4.0 mg/kg, High Molecular Weight, InVitroGen, San Diego, CA) via the tail vein. This procedure took an average of 10 minutes per animal and care was taken to ensure the saline treated dams were anaesthetized for the same length of time as the PolyI:C treated dams. Weight and temperature were measured again 8, 24, and 48 h after the injection. Dams were otherwise left undisturbed until the day after parturition. Experimenters were blind to the treatment of the dams and pups during the course of all experiments. The day of parturition was designated postnatal day (PND) 0. On PND 1, litters were weighed and culled to 10 pups per litter (6 males and 4 females where possible). Observations of maternal behaviour were initiated the next day and continued for 7 days, with two 10 min sessions twice daily (0800-1000h and 1500-1700h). A maternal behaviour rating scale was adapted from previous studies (Barha et al., 2007). The maternal behaviours recorded

were: 1) arch-back nursing/licking and grooming (ABN/LG): mom over the pups in an arched position and licking them; 2) blanket nursing: mom lying over the pups, relaxed; 3) passive nursing: mom lying on her side nursing the pups; 4) off-nest: mom outside of nest and away from the pups. Other than maternal observations and routine husbandry (including taking litter weights on PND8 and 14), litters were left undisturbed until weaning on PND 21. Weaned pups from the same litter were housed in same-sex cages of 3 or 4 in separate colony rooms. On PND 53, 1 or 2 pups of each sex from each litter were randomly selected for the present experiment. Rats were individually housed and food restricted to 85% of their free feeding weight until PND 60 when lever training commenced. Pups were randomly selected from a subset of litters and no more than 2 pups of the same sex from the same litter were included in the current experiment. This was done to control for any potential litter effects. Roughly 20 pups were taken from each treatment group because this number is sufficient to allow the detection of a significant difference between the groups, if it exists. The remaining pups from each litter were used for other studies which were not part of this thesis.

Estrous cycle measurements

From PND 56 to the last day of experimental testing, vaginal smears were taken for each female offspring (n=22). Every morning between 08:30 and 10:00 h, a vaginal sample was collected by inserting a plastic pipette tip loaded with 20 μ l of 0.9% saline into the vagina (~5 mm deep). The saline was ejected, immediately reloaded into the pipette, and then ejected onto a glass slide. Typically, 5 samples were put on a given slide, after which Cytoprep (Fisher Scientific) was sprayed onto the slide. Wet samples were viewed using a light microscope and estrous cycle was determined using established cytology methods (Goldman et al., 2007; Marcondes et al., 2002). Four phases were identified: 1) proestrus: majority of the cells present

are round, nucleated epithelial cells; 2) estrus: majority are irregular, not nucleated cells; 3) metestrus: consists of equal number of nucleated epithelial, non-nucleated cells, leukocytes in the sample; and 4) diestrus: majority of cells are leukocytes. The estrous cycle begins with the proestrus stage, where follicles begin to grow, with high levels of estrogen and progesterone secretion (Goldman et al., 2007). Estrus stage is where the female rat is the most receptive, with levels of progesterone the highest and estrogen the most influential (Goldman et al., 2007). Metestrus (i.e., diestrus 1) stage is where low levels of progesterone is secreted and diestrus (i.e., diestrus 2) is where progesterone is being secreted again, as the body prepares for pregnancy. Female rats in both treatment groups displayed normal alterations in the stage of the estrous cycle during the period prior to behavioural testing. During testing, no effort was made to control for the stage of the estrous cycle; rather, performance was correlated with the naturally occurring stage of the estrous cycle. No correlation was observed between stage of the estrous cycle and trials to criterion (TTC) for either the visual cue or set-shifting test day in either treatment group (Pearson r 's < 0.20, p 's > 0.48 for visual cue and set-shifting). A significant correlation was observed between stage of estrous cycle and TTC on reversal learning day in the saline treated offspring ($r = -0.677$, $p = 0.045$) but not the PolyI:C treated offspring ($r = 0.04$, NS). TTC were greater for females in metestrus and diestrus ($n = 3$, $TTC = 107.67$) than proestrus and estrus ($n = 6$, $TTC = 80.83$) in the saline-treated group. The importance of this observation is not clear; however, it is worth noting that the group means for TTC on the reversal learning day were not different between the two treatment groups (see Results, Reversal learning). In addition, the small sample size ($n = 9$ observations) makes drawing firm conclusions from this correlation difficult. Despite the small sample size, the significant

correlation for the saline female rats shows that estrous cycle may play a role during reversal learning day.

Operant Chamber Apparatus

All training and testing were conducted in four operant chambers (32 x 25.5 x 25 cm; MedAssociates Systems, St. Albans, VT, USA). Each operant chamber was located within a wooden sound-attenuating box (63.6 x 35.6 x 75.6 cm; W x H x L) equipped with a fan that provided background noise and ventilation. Each operant chamber included a food receptacle where food rewards (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, Frenchtown, NJ) were delivered via a pellet dispenser. A retractable lever and a stimulus light were positioned on either side of the receptacle. A single house light (100 mA) located near the top and center of the wall opposite the levers illuminated the chamber. The chamber floor consisted of a removable metal grid which contained a detachable tray. A personal computer and interface box controlled the presentation of the trials and recorded all experimental data.

Lever training

Pre-training protocols closely followed those previously published (Floresco et al., 2008; Floresco et al., 2009). Rats were given 20 reward pellets in their home cages the day prior to the commencement of lever training. On the first day of training, ~2-3 reward pellets were crushed into a fine powder and placed on the extended lever. Rats were trained under a fixed-ratio 1 (FR1) schedule to a criterion of 45 presses in 30 min. Rats were first trained on one lever before being trained on the other (counterbalanced left/right between subjects). On subsequent days, rats were familiarized with the insertion of levers into the chamber and trained to press them within 10 s of insertion. These training sessions consisted of 90 trials and began with both levers retracted and the chamber in darkness. A trial began every 20 s with the illumination of the

house light and the insertion of one of the levers into the chamber. If the rat failed to press the lever within 10 s, the lever was retracted, the house light was extinguished, and the trial was scored as an omission. If the rat responded within 10 s, the lever was retracted, a reward pellet was delivered, and the house light remained on for 4 additional seconds. The left and right levers were each presented once in every pair of trials with the order of presentation randomized within each pair. The stimulus lights above the levers were never illuminated during lever training sessions. The criterion to pass training was to make ≤ 5 omissions in a training session. Rats were trained in this manner for a minimum of 5 days or until the criterion was reached. Upon reaching criterion, the side preference of the rat was determined. Here, both levers were inserted and pressing either one would result in the delivery of a reward pellet. On subsequent trials, rats were required to alternate between left and right levers to obtain a reward. The session ended after 7 reward pellets were delivered. A rat's side preference was determined using the total number of left and right lever presses. If the total right and left lever presses were comparable, then the lever chosen first was counted as the side preference. However, if a rat consistently focused on one lever during the session ($\geq 2:1$ ratio) then that side would be taken as the side preference. Testing began the day following the final training session.

Visual-cue discrimination

The initial discrimination required rats to press the lever with the stimulus light illuminated above it. Rats were trained to a criterion of 10 consecutive correct choices. Each daily session consisted of a minimum of 30 trials and a maximum of 150 trials. If a rat reached criterion before 30 trials were conducted, the program terminated after 30 trials. If a rat did not reach criterion within 150 trials, visual-cue discrimination testing continued on subsequent days until criterion was reached. Each session began with both levers retracted and the chamber in

darkness (the inter-trial state). Every 20s, a trial began with one of the stimulus lights being illuminated. The house light was illuminated 3 s later with both levers being inserted into the chamber. A correct response (pressing the lever underneath the illuminated stimulus light) resulted in the retraction of both levers and the delivery of a food pellet. The house light remained on for 4 s before being extinguished and the chamber was returned to the inter-trial state. An incorrect response (pressing the lever not underneath the illuminated stimulus light) resulted in the chamber immediately reverting to the inter-trial state and no food reward being delivered. Failure to respond to either lever within 10 s also resulted in the chamber returning to the inter-trial state with the trial being scored as an omission. The left and right stimulus lights were illuminated once in every pair of trials with the order within each pair randomized. The lever the rat chose and the stimulus light illuminated were recorded for each trial. Omissions were not included in the TTC.

Shift to response discrimination (strategy set-shift)

On the day following visual-cue discrimination testing, rats were required to ignore the visual-cue (illuminated stimulus light) and respond to a spatial cue (the lever opposite to their side preference) to receive a reward pellet. Trial format and criterion measures were the same as the visual cue discrimination day. Errors during this phase of testing were broken down into 3 subtypes as has been previously described (Floresco et al., 2008; Floresco et al., 2009; Haluk & Floresco, 2009; Ragozzino, 2002). Perseverative errors were scored when a rat continued to use the previously relevant, but currently irrelevant strategy. For example, if a rat pressed the lever opposite to the side they required to respond while the stimulus light above it was illuminated, a perseverative error was counted. Eight out of every 16 consecutive trials (defined as a block) allowed the rat to respond this way. For each block, perseverative errors were counted when rats

pressed the incorrect lever 6 or more trials per block. Once a rat made 5 or fewer incorrect lever presses during a block, all subsequent perseverative errors were counted as regressive errors. Perseverative errors are considered a measure of disengaging from a strategy while regressive errors are commonly considered a measure of maintaining a new strategy (Floresco et al., 2008; Floresco et al., 2009; Haluk & Floresco, 2009; Ragozzino, 2002). Never-reinforced errors were scored when rats pressed the incorrect lever when the stimulus light was illuminated above the correct lever.

Response reversal

During this phase of testing, rats were required to press the lever opposite to the one required during the previous set-shifting day (e.g., press left lever on set-shift day then press right lever on reversal day). The trial format and criterion were identical to the visual-cue and set-shifting test days. Errors were broken down into 2 subtypes. Perseverative and regressive errors were counted in a similar manner to the set-shifting day. However, perseverative errors were scored when a rat made 10 or more errors within a block of 16 trials, excluding the first block when errors were always scored as perseverative (Floresco et al., 2009). All subsequent errors (i.e., once less than 10 errors per block were made) were scored as regressive errors.

Data analysis

All analyses were computed using SPSS Version 18. Effects of PolyI:C treatment on the pregnant dams and litters were analyzed with repeated measures ANOVA using Treatment (saline vs. PolyI:C) as a between subjects factor and Time (0, 8, 24, 48 h post-treatment) as a within subjects factor. Data related to operant behaviour testing were analyzed with two-way ANOVA/ANCOVA with Treatment and Sex as between subjects factors for each test day and average response latency as a covariate where appropriate. Simple main effects were tested with

independent samples t-tests. Errors during behavioural testing were analyzed with repeated measures ANOVA using Error Type as a within subjects factor and Treatment and Sex as between subjects factors. Post-hoc tests for the repeated measures ANOVA were performed by hand using the Newmann-Keuls method and P values of < 0.05 were considered significant.

RESULTS

Effects of PolyI:C on dams and pups:

Pregnant dams were randomly assigned to receive either PolyI:C (n=11) or saline (n=9). Weight and rectal temperature were taken at 0, 8, 24, and 48 h after PolyI:C injection. Statistical analysis of dam weights revealed a significant main effect of Time ($F(3, 54) = 37.38, p < 0.001$), a significant Treatment by Time interaction ($F(3, 54) = 11.56, p < 0.001$), and no main effect of Treatment ($F(1, 18) = 2.47, NS$). Post-hoc analyses revealed that dam weights differed between the saline (0 h: 361.44 ± 13 g; 8 h: 352.09 ± 12 g) and PolyI:C treated groups (0 h: 397.02 ± 8 g; 8 h: 377.55 ± 8 g) at 0 and 8 h ($p < 0.05$). Further analysis of the weight data revealed that dams in both groups lost weight in response to being anesthetized, although the PolyI:C treated animals lost more weight (Figure 1A; saline: 2.52%; PolyI:C 4.93%). At 24 and 48 h, the PolyI:C treated animals gained significantly less weight than the saline treated animals, as revealed by a significant Time by Group interaction for the percentage of weight lost at 8, 24, 48 h relative to initial weight at 0 h ($F(2, 36) = 5.62, p < 0.007$; post-hoc for 24, 48 h, $p < 0.05$). Analysis of temperature data (Figure 1B) showed a significant Treatment by Time interaction ($F(3, 54) = 6.41, p = 0.001$) without significant main effects of Treatment ($F(1, 18) = 0.54, NS$) or Time ($F(3, 54) = 2.68, NS$). Post-hoc analyses revealed that the temperature of the PolyI:C treated animals was significantly higher than the saline treated animals 8 h after injection. An average of 13.89 ± 1 and 12.55 ± 1 pups were born to the saline treated and PolyI:C treated dams,

respectively. No significant effect was noted for prenatal treatment regarding the number ($t(18) = 0.77$, NS) or weight of the pups on PND 1, 8, 14, or 21 (data not shown; $F(3, 54) = 0.59$, NS).

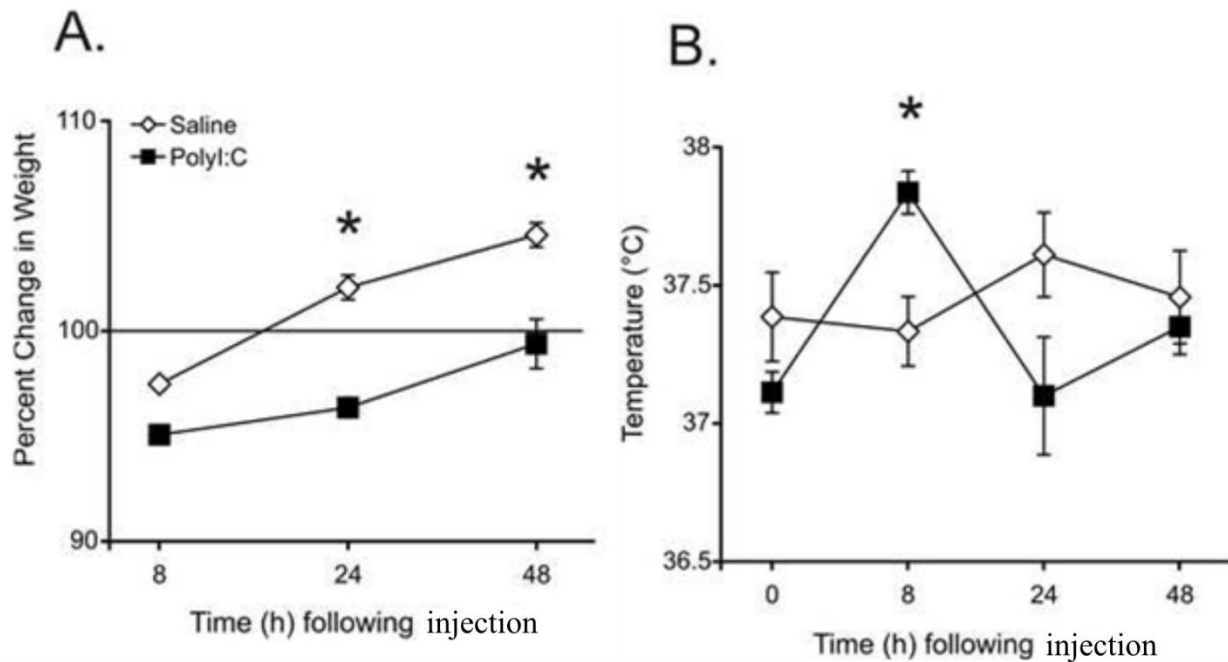


Figure 1. A. Effects of prenatal treatment on weight of pregnant dams (gestational day 15; Saline: $n = 9$; PolyI:C: $n = 11$). Weight change is expressed as a percentage of baseline weight at 0 h immediately prior to treatment. Dams treated with saline gained significantly more weight than those treated with PolyI:C at the 24 and 48 h time points. **B.** Effects of treatment on rectal temperature of dams. PolyI:C significantly increased rectal temperature of the dams 8, but not 24 or 48, h after treatment. All values are means \pm SEM. * denotes $p < .05$.

Maternal behaviour

Maternal behaviour was measured twice daily from PND 2 to 8 during the light cycle. Measures reported include ABN/LG and off nest time. Blanket and passive nursing were infrequently observed in our sample. When observations from all sessions were summed, saline treated dams displayed $2,735.56 \pm 480$ s of ABN/LG and $3,921.67 \pm 385$ s off nest time. PolyI:C treated dams displayed $3,077.27 \pm 315$ s of ABN/LG and $3,365.46 \pm 353$ s off nest time. A repeated measures ANOVA revealed no effect of Treatment ($F(1, 18) = 0.23$, NS), Day ($F(6, 108) = 1.26$, NS) or a Treatment x Day interaction ($F(6, 108) = 1.77$, NS) on ABN/LG. Similarly, no effect of Treatment ($F(1, 18) = 0.87$, NS), Day ($F(6, 108) = 1.91$, NS) or a Treatment x Day interaction ($F(6, 108) = 0.87$, NS) was found for off nest time.

Operant lever training

The effects of treatment and sex on the number of days required for operant training was considered. Inspection of the data revealed that female rats took longer to complete training regardless of their treatment condition (days of FR1: males 3.32 ± 0.6 , females 4.09 ± 0.3 ; days of retractable lever: males 5.32 ± 0.1 , females 6.09 ± 0.3 ; omitted trials: males 1.55 ± 0.4 , females 3.00 ± 0.4). These differences were significant for the number of days required for retractable lever training ($F(1, 40) = 4.84$, $p = 0.034$) and omitted trials during the last training day ($F(1, 40) = 7.73$, $p = 0.008$). No significant effect of sex was observed for FR1 training ($F(1, 40) = 2.83$, NS) and no effect of PolyI:C treatment or interactions between sex and PolyI:C treatment were found for days required for FR1 training, retractable lever training, or omitted trials (all F 's < 1.1 , NS).

Visual-cue discrimination

The number of TTC and errors were similar for all groups during the visual-cue discrimination day (Figures 2A, B). These impressions were confirmed statistically, with non-

significant effects of Treatment (TTC: $F(1, 40) = 0.28$, NS; Errors: $F(1, 40) = 0.57$, NS), Sex (TTC: $F(1, 40) = 2.25$, NS; Errors: ($F(1,40) = 2.74$, NS), and Sex x Treatment interactions (TTC: $F(1, 40) = 0.22$, NS; Errors: $F(1,40) = 1.05$, NS). While average response latencies (ARL) did not differ as a result of treatment ($F(1,40) = 0.17$, NS), they differed for sex with female rats taking significantly longer than males to respond when the levers were inserted (Figure 2C; $F(1, 40) = 4.70$; $p = 0.03$). Taken together, these results suggest that PolyI:C treatment did not affect the learning of visual-cue discrimination, although females rats, regardless of treatment condition, were slower than male rats to respond to insertion of the levers.

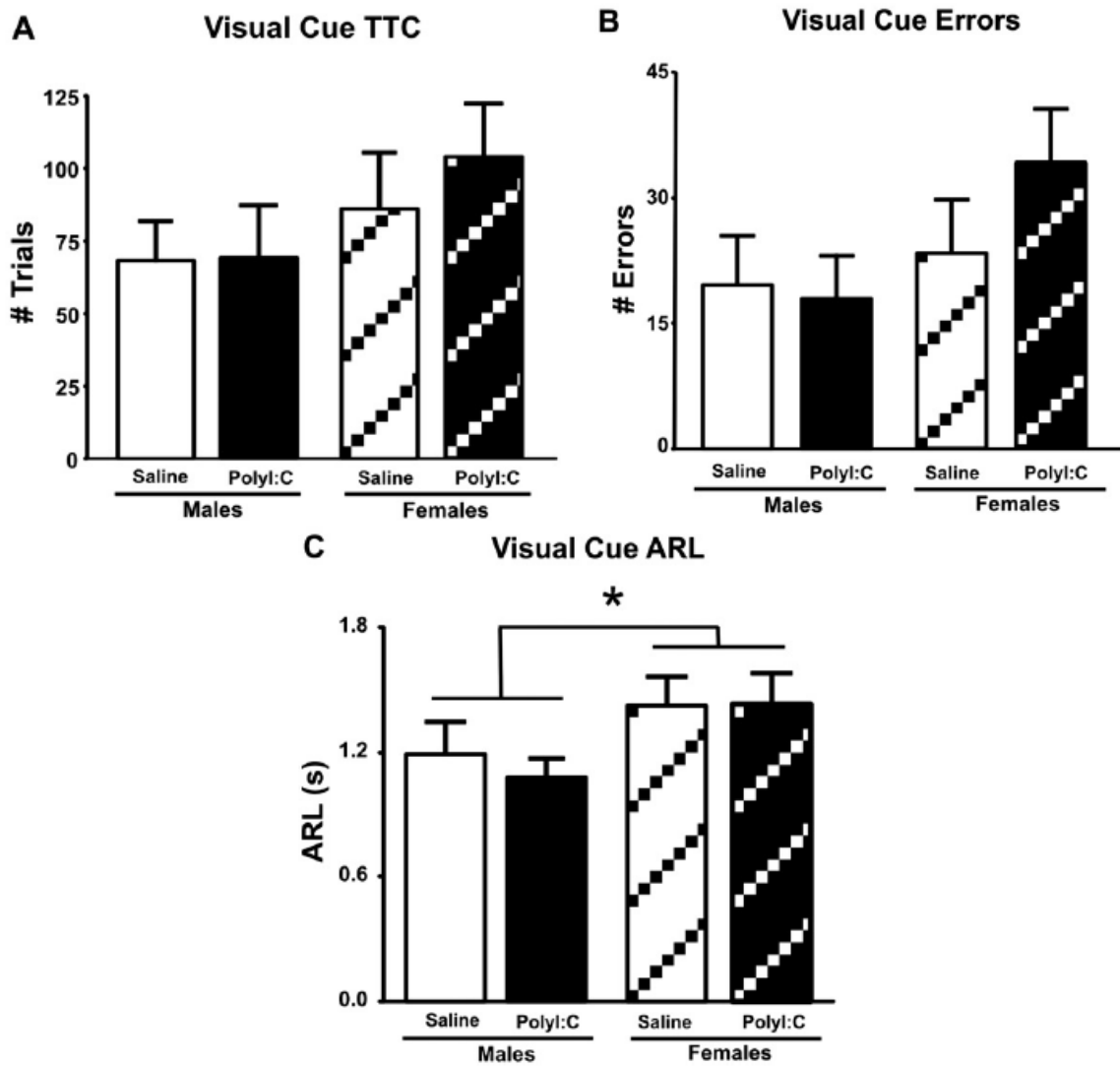


Figure 2. Performance of rats in the PolyI:C or saline-treated groups on the visual cue discrimination day. **A.** Trials to criterion (TTC). **B.** Total errors. **C.** Average response latencies (ARL; s) to press a lever. Female rats had significantly increased ARL's regardless of treatment. Saline: n = 11 males, 10 females; PolyI:C: n = 11 males, 12 females. * denotes $p < 0.05$.

Shift to response discrimination

On this test day, rats were required to shift their strategy from responding to the light cue to using an egocentric spatial strategy. Inspection of the data (Figure 3A) revealed that males treated prenatally with PolyI:C required more TTC (102.46 ± 12 trials) than males treated prenatally with saline (64.73 ± 8 trials). In contrast, female rats performed similarly regardless of treatment (PolyI:C group: 80.92 ± 10 trials; saline group: 82.50 ± 13). A two-way ANOVA revealed non-significant main effects of Treatment ($F(1, 40) = 2.90$; NS) and Sex ($F(1, 40) = 0.03$; NS) while a trend toward a significant Sex X Treatment interaction was observed ($F(1, 40) = 3.43$; $p = 0.07$). Further analysis of the data for each sex revealed that, when considered alone, the male PolyI:C treated offspring took significantly more trials to reach criterion ($t(20) = -2.71$, $p = 0.01$). No difference for TTC was observed when the females were considered alone ($t(20) = 0.01$, NS). Average response latencies were significantly slower for the female rats ($F(1, 40) = 7.55$, $p = 0.009$) with no significant main effect for Treatment ($F(1, 40) = 1.37$, NS) or a Sex x Treatment interaction ($F(1, 40) = 0.37$, NS). Due to a significant sex difference for ARL, analysis of treatment effects on TTC with ARL as a covariate was performed. This analysis showed that PolyI:C male rats were significantly slower in reaching criterion than saline-treated male rats ($F(1, 19) = 5.209$; $p = 0.03$) whereas females rats required a similar number of TTC regardless of treatment ($F(1, 19) = 0.03$; NS). The animals did not differ on the total number of errors (Figure 3B) made when either Treatment ($F(1, 40) = 2.33$, NS) or Sex ($F(1, 40) = 0.03$, NS) were considered; however, there was a significant Sex by Treatment interaction ($F(1, 40) = 4.50$, $p = 0.04$). Male PolyI:C treated offspring made significantly more errors (post-hoc, $p < 0.05$) than saline-treated offspring whereas no significant difference existed for female offspring. When

errors are broken down into perseverative, regressive, and never-reinforced subtypes, a repeated measures ANOVA revealed no main effect of Sex ($F(2, 80) = 0.30$, NS) or Treatment ($F(2, 80) = 0.58$, NS) but a significant Sex by Treatment by Error interaction ($F(2, 80) = 4.15$, $p = 0.02$). Post-hoc comparisons indicated that PolyI:C treated males committed significantly more perseverative errors than saline-treated males (Figure 3C; $p < 0.05$). No significant differences were noted for regressive or never-reinforced errors in the male rats or any of the error subtypes for the female subjects (Figure 3D). Hence, when compared to the saline-treated males, PolyI:C treated males displayed impaired set-shifting as a result of increased perseverative errors.

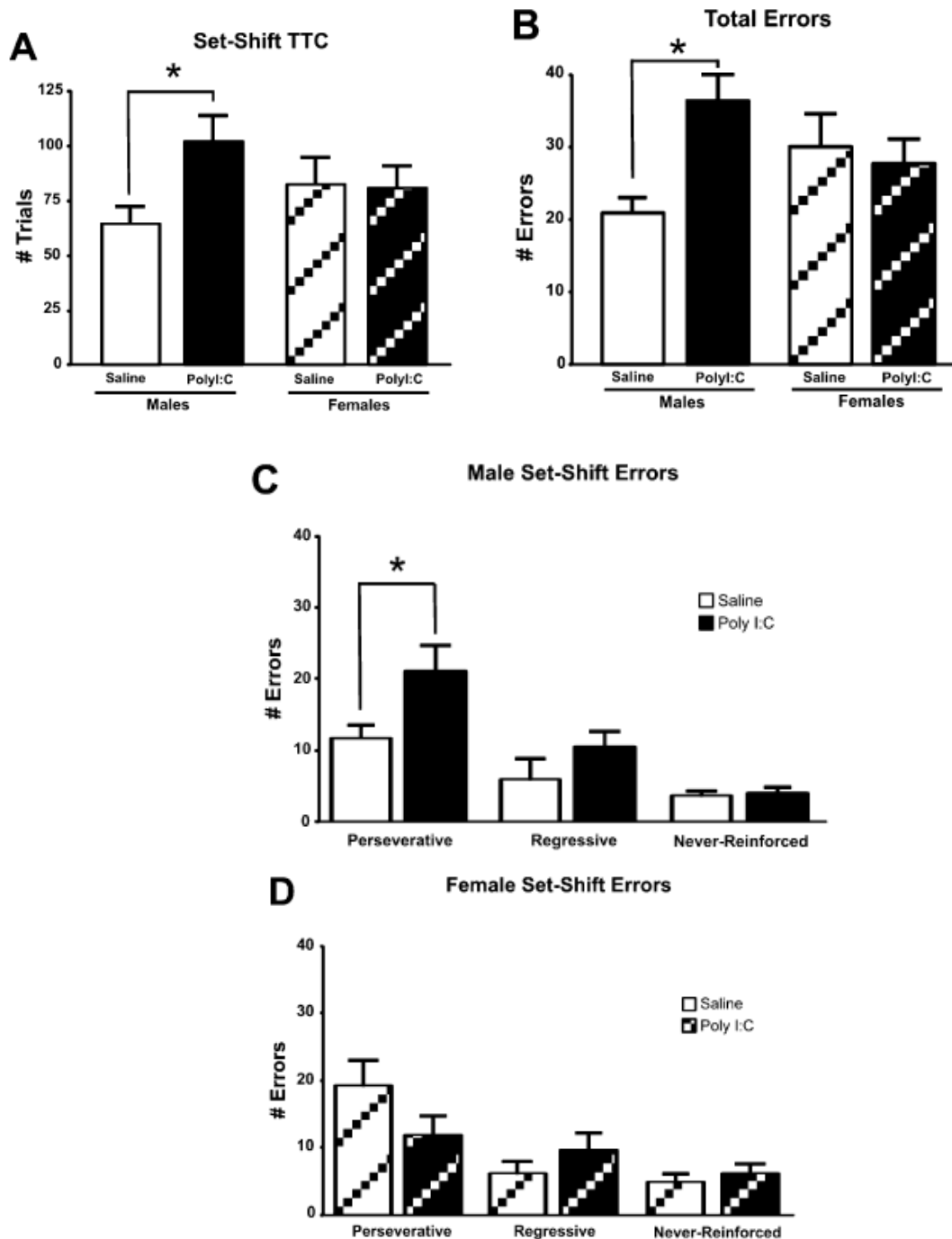


Figure 3. Effects of prenatal PolyI:C or saline treatment on set-shifting from a visual to response based strategy. **A.** Effects of prenatal PolyI:C treatment on trials to criterion (TTC). **B.** Total errors committed during set-shifting. Male offspring prenatally treated with PolyI:C took significantly more trials to reach criterion and made significantly more total errors than saline treated offspring. **C.** Error subtypes committed by the males. The impairment in set-shifting for the PolyI:C-treated males was due to a significant increase in perseverative errors. **D.** Error subtypes committed by the females. * denotes $p < 0.05$.

Reversal learning

PolyI:C treated male offspring required fewer TTC (Figure 4A, 74.00 ± 6 trials) than saline-treated male adult offspring (104.00 ± 18) whereas the female rats showed similar performance regardless of treatment (PolyI:C group: 95.50 ± 6 trials; saline group: 91.40 ± 9 trials). ANOVA revealed that these differences were not significant for either TTC (Sex: $F(1, 40) = 0.17$; Treatment: $F(1, 40) = 1.41$; Sex x Treatment interaction: $F(1, 40) = 2.45$, all NS) or total errors (Sex: $F(1, 40) = 0.45$; Treatment: $F(1, 40) = 0.36$; Sex x Treatment interaction: $F(1, 40) = 0.77$, all NS). Similar to the other test days, females were significantly slower to respond to insertion of the levers than males regardless of treatment condition ($F(1, 40) = 11.11$, $p = 0.002$). An ANCOVA performed on TTC with ARL as the covariate revealed the same non-significant effects noted above (statistics not shown). Analysis of error subtype revealed a significant Sex x Treatment x Error interaction ($F(1,40) = 4.10$, $p = 0.049$) without significant main effects for Treatment ($F(1, 40) = 0.03$, NS) or Sex ($F(1, 40) = 0.21$, NS). PolyI:C treated male offspring made significantly fewer regressive errors than saline-treated male offspring (Figure 4B; $p < 0.05$) while the female groups did not differ (Figure 4C). Therefore, during reversal learning, PolyI:C treated male rats made fewer regressive errors than saline-treated males, whereas female adult offspring performed similarly regardless of treatment.

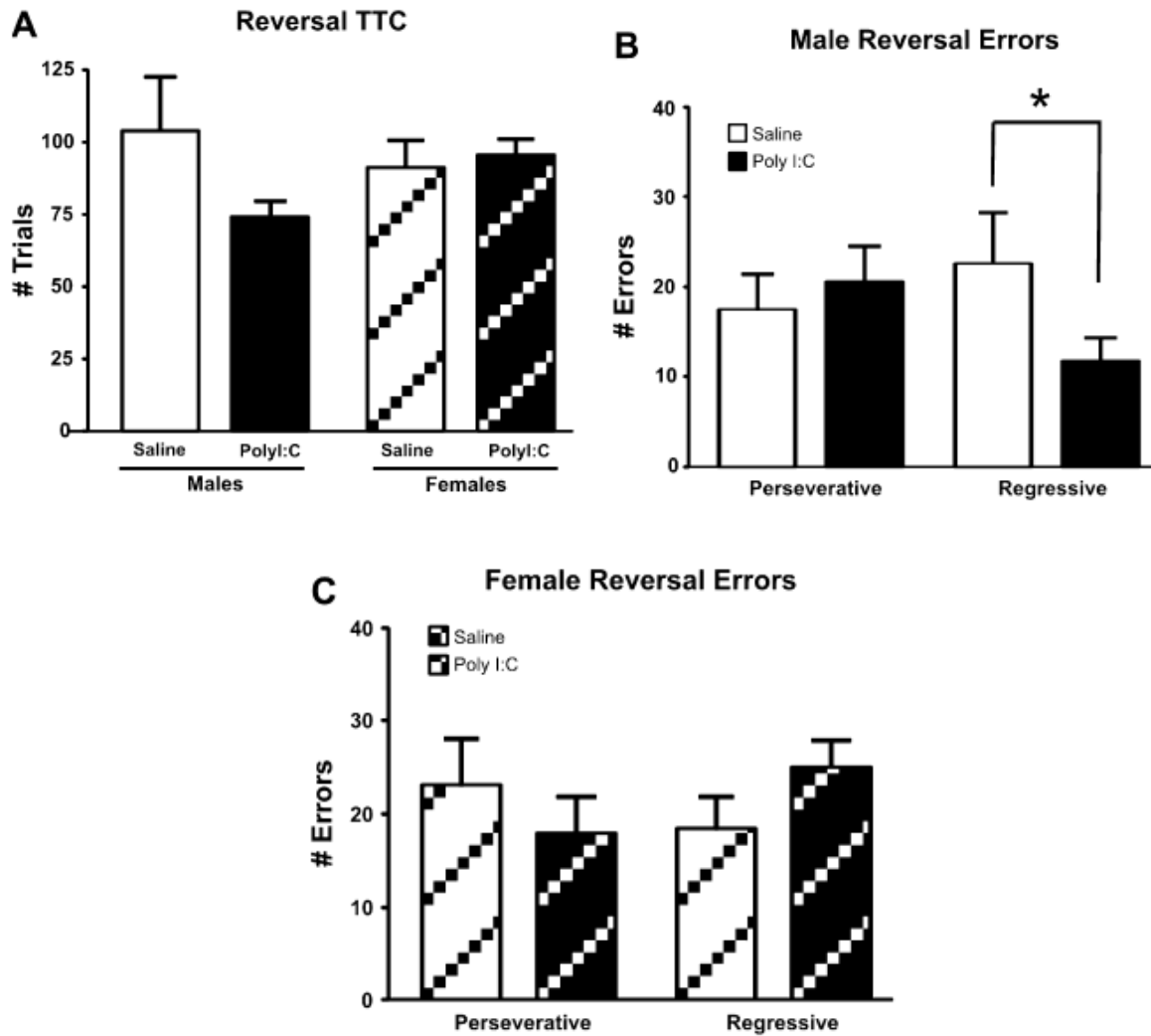


Figure 4. Effects of prenatal PolyI:C or saline treatment reversal learning. **A.** Effects of prenatal PolyI:C treatment on trials to criterion (TTC). **B.** Analysis of the subtypes of errors made by the male animals. Male PolyI:C-treated offspring made significantly more regressive errors during the reversal learning phase of the task. **C.** Subtypes of errors made by the females. * denotes $p < 0.05$.

DISCUSSION

The data generated from the current study supports the hypothesis that prenatal PolyI:C treatment alters cognition in the adult offspring, specifically set-shifting. PolyI:C treated male offspring were impaired during the set-shift day, where they took more trials to reach criterion than the saline treated male offspring (Figure 3A). The increase in trials was a result of a significant increase in total errors (Figure 3B) and perseverative errors (Figure 3C). On reversal learning day, PolyI:C treated male offspring took fewer trials to reach criterion (Figure 4A), with a significant reduction in the number of regressive errors (Figure 4B). Again, no differences were observed in the PolyI:C treated female adult offspring (Figure 4C) in reaching trials to criterion on this day. No differences were observed during the visual-cue discrimination day, as the number of trials to reach criterion did not differ between groups. However, all female rats took more time to respond to the lever compared to male rats (Figure 2C). The acquisition phase of lever training showed that female adult offspring took more days to complete training and omitted more trials on the last day of training, when compared to the male adult offspring. On the day of the PolyI:C injection, acute PolyI:C treatment significantly decreased the weight of dams at 8h. However, PolyI:C dams had a lower weight before the treatment (e.g., 0h), compared to the saline treated dams. The PolyI:C treated dams also gained significantly less weight than the saline treated dams at 24h and 48h (Figure 1A). In addition, PolyI:C treatment increased the temperature of the dams at 8h, whereas no change was observed in saline treated dams (Figure 1B). Despite the changes induced by the acute PolyI:C treatment in treated dams, pup litter size and weight were not significantly different from saline treated dams. Maternal behaviour observations yielded no significant difference between the two groups.

Effects of PolyI:C on Set-shifting Day

Acute prenatal PolyI:C treatment disrupted the ability to set-shift from a visual-cue to a response discrimination in the male, but not female adult offspring (Figure 3A). PolyI:C male offspring took significantly more trials to reach criterion, as this was driven by an increase in total errors, specifically perseverative errors (Figure 3B). PolyI:C treated male offspring continuously followed the stimulus light when it was no longer the rewarding strategy. This perseveration is consistent with mPFC dysfunction (Birrell & Brown, 2000; Floresco et al., 2008). As mentioned earlier, the mPFC is a key player in mediating set-shifting (Floresco et al., 2008). Inactivation to this area impaired set-shifting in the T-maze task, whereby lesioned rats made significantly more perseverative errors (Floresco et al., 2008). In addition, damage to the subcortical regions and its circuitry back to the PFC induce similar set-shifting impairments (Block et al., 2006; Haluk & Floresco, 2009). Block et al. (2006) found that bilateral inactivation of the mediodorsal nuclei of the thalamus (MD), of the rat, impaired set-shifting and increased the number of perseverative errors, on the T-maze. In the same study, unilateral inactivation to one side of the MD and inactivation to the contralateral PFC disrupted set-shifting in a similar manner. In addition, the same type of unilateral inactivation to the PFC and nucleus accumbens (NAc) impaired set-shifting as well (Block et al., 2006). Prenatal PolyI:C treatment may have altered the development of the mPFC and its connections with the NAc and MD in the fetal brain; hence, impairing the adult offspring's ability to set-shift.

Furthermore, the dopaminergic system in both the mPFC and NAc may have played a role in disrupting set-shifting in the PolyI:C treated adult offspring. Local injection with eticlopride (i.e., D₂ antagonist), or PD-168,077 (i.e., D₄ agonist) to the mPFC of the adult rat increased perseveration when set-shifting from either a visual-cue to response discrimination or

vice versa (Floresco et al., 2006a). Microinfusions of quinpirole (e.g., D₂ agonist) administered into the rat NAc also increased perseverative errors (Haluk & Floresco, 2009). Prenatal PolyI:C treatment has been shown to increase DA sensitivity in the offspring (Meyer & Feldon, 2010). Zuckerman and colleagues (2003) showed that prenatal treatment with 5mg/kg of PolyI:C increased locomotor activity in the rat adult offspring when amphetamine was given. Furthermore, amphetamine increased DA release in the striatum, using an *in vitro* preparation (Zuckerman et al., 2003). Both Meyer et al, (2008a) and Winter et al., (2008) found that PolyI:C treated mice offspring had increased levels of DA in the mPFC and NAc. Hence, prenatal PolyI:C treatment may alter the developing dopaminergic system in the mPFC and its subcortical regions by altering DA levels and receptor subtypes. As a result, these changes induced the inability to disengage from a previous rewarding but currently non-rewarding strategy in the adult offspring, impairing set-shifting.

Dopaminergic system may not be the only system affected by the prenatal treatment, as NMDA receptors in the mPFC also play a significant role in mediating set-shifting. Disruptions in the GABAergic and NMDA-R systems have been frequently seen in prenatal infection studies (Belforte et al., 2010; Meyer & Feldon, 2010; Zuckerman et al., 2003). Administration of MK-801 induced hyper locomotion in prenatally treated PolyI:C rodents (Meyer & Feldon, 2010). Prenatal PolyI:C treatment also increased GABA_A and decreased NMDA receptors (Meyer & Feldon, 2010). The decrease in NMDA receptors may inhibit the release of GABA; therefore, result in an upregulation of GABA_A receptors. This in turn may compensate for the reduced GABA levels in the brain. The change in both NMDA and GABA receptors, as a result of prenatal PolyI:C treatment, may induce set-shifting impairments in the PolyI:C treated offspring. Stefani and Moghaddam (2005) found that infusion of a NMDA antagonist to the PFC increased

perseverative errors in the T-maze. Rodefer et al, (2007) found that subchronic treatment with ketamine impaired EDS during the digging bowl task, which increased perseverative errors. It is believed that the NMDA receptor blockade result in the inhibition of GABA interneurons, which in turn disinhibits glutamate receptors, leading to increased activity of the PFC and its subcortical regions (Belforte et al., 2010). In addition, GABA_A receptor blockade in the mPFC of the rat induced set-shifting but not reversal learning deficits in the operant chamber task (Enomoto et al., 2011). During this task, the GABA_A antagonist was either microinfused the day before or 30 mins before the set-shifting test. Microinjecting the antagonist the day before increased perseverative errors. Interestingly, the same antagonist administered 30 min before set-shifting increased never-reinforced errors (Enomoto et al., 2011). This data illustrates that the time of disruption plays a critical role in inducing cognitive impairments. Given that the increase in perseverative errors were only present when the GABA_A antagonist was applied the day before and not 30 mins before set-shifting, it shows that disruptions occurred earlier in time increase perseverative errors (Enomoto et al., 2011). From this, it supports the notion that prenatal PolyI:C treatment may have induced long-lasting changes in the fetal brain and as a result, induced impairments in the adult offspring during set-shifting. Furthermore, prenatal treatment may induce alterations to the NMDA receptors in the fetal frontal cortex. This, in turn may affect the GABAergic system, resulting in perseveration in the adult offspring. However, given that the current study did not examine neurotransmitter levels and receptor density, these explanations are only speculative. More studies examining the neurotransmitter systems are needed.

Effects of PolyI:C on Reversal Learning Day

PolyI:C treated male offspring showed a tendency to take fewer trials to reach criterion compared to the saline treated male offspring (Figure 4A). Females, on the other hand, did not differ due to treatment. The decrease in the number of trials to reach criterion during reversal learning is consistent with the results shown in Zuckerman and Weiner (2005). They found that acute injection of PolyI:C on GD 15 or 17 facilitated the reversal component of the Morris water T-maze. Here, adult offspring were trained to always swim into one of the two arms to find the hidden platform. On the test day, rats were initially re-exposed to the arm in which the hidden platform was located previously; however, after 5 consecutive correct choices, the platform was moved to other arm. Both groups of PolyI:C treated rats took significantly fewer trials to reach criterion during reversal learning (Zuckerman & Weiner, 2005). However, prenatal PolyI:C treatment has also been shown to impair reversal learning (Meyer et al., 2006a). This discrepancy between data sets may be a result of different species, given that the latter study used mice. Furthermore, Meyer and colleagues, (2006a) used 5mg/kg, as opposed to 4mg/kg. A higher dose may induce a more prominent deficit in the treated adult offspring. Task trials and protocol were also slightly different between the two studies and the current study. Meyer and colleagues (2006a) had trained rats to a criterion of 11 correct arm entries for 2 consecutive days, whereas Zuckerman and Weiner (2005) had a criterion of 5 consecutive correct choices in one day. Hence, effects of PolyI:C may be sensitive to the protocol used, dose of drug and the species tested, resulting in contradictory findings between studies. Nevertheless, given that the current study utilized the same dosage and species as Zuckerman and Weiner (2005), it is reasonable to conclude that a facilitation during reversal learning, as a result of prenatal PolyI:C treatment in the rat, has reliability. However, since all rats were tested from set-shifting to

reversal learning day, it could be that prenatal treatment with PolyI:C may facilitate the second cue-dimension or strategic shift (e.g., test day 2), regardless of whether it is reversal learning or not. Thus, more studies are needed to clarify this potential facilitation.

PolyI:C male offspring made significantly fewer regressive errors compared to the saline treated male offspring (Figure 4B). The decrease in regressive error is characteristic of NAc dysfunction, specifically the NAc shell. Floresco et al. (2006b) found that inactivation of the NAc shell decreased regressive errors during a set-shift from a response to visual-cue discrimination on the T-maze task. Disruption to the NAc shell seems to impair the ability to learn the irrelevance of a non-rewarding stimulus; hence, improving performance in acquiring a new strategy when the old strategy was no longer reinforced. In the current study, PolyI:C male rats attended to the rewarding stimulus much more rapidly than controls. Prenatal treatment with PolyI:C may have induced alterations to the NAc shell during fetal development.

The decreased number of regressive errors may also reflect latent inhibition (LI) impairments. LI is the retarded acquisition of a previously non-reinforced, but currently reinforced stimulus. Studies have shown that prenatal PolyI:C treatment reduced LI, in that avoidance learning was not disrupted, when compared to saline treated offspring (Zuckerman et al., 2003; Zuckerman & Weiner, 2005). A study conducted by the same group found that electrolytic lesions to the NAc shell abolished LI, whereby shell lesioned rats very rapidly incorporated the initial irrelevant tone as being relevant. The facilitated learning of the previously irrelevant but currently relevant stimulus is consistent with the current data, as the treated male offspring were faster to acquire the previously irrelevant but currently relevant strategy. Therefore, it is possible that the decrease in regressive errors exhibited by the treated offspring may be reflective of LI impairment, potentially linked to NAc shell damage.

In addition, disruption in 5-HT receptors may also facilitate reversal learning. Boulougouris and Robbins, (2010) found that microinfusions of 5-HT_{2c} antagonist to the OFC, but not mPFC or NAc, improved reversal learning in an operant serial reversal learning task. This antagonism significantly reduced the number of errors in the treated animals. Interestingly, 5-HT_{2A} antagonist impaired reversal learning (Boulougouris & Robbins, 2010). Therefore, it seems that 5-HT receptor subtypes in the OFC have differential roles in mediating reversal learning. Prenatal PolyI:C treatment may affect the 5-HT_{2C} receptor subtype; hence, facilitating reversal learning in the current experiment. However, more prenatal infection studies are needed to examine the density and levels of 5-HT in the PFC and limbic systems to understand how this system is affected, if at all.

Effects of PolyI:C Treatment on Visual-cue Discrimination Day

No difference was observed between the male saline and PolyI:C treated adult offspring in reaching trials to criterion. In addition, male saline treated adult offspring performed in a similar manner as rats from studies utilizing the same operant based task (Floresco et al., 2009). In the Floresco and colleagues (2009) paper, the average number of trials to reach criterion for the saline rats were around 39 ± 9 . This is comparable to the current data (e.g., 57 ± 14). The number of TTC on subsequent test days (e.g., set-shifting and reversal learning days) was also in parallel with the data from the Floresco and colleagues (2009) study. In addition, the dams used in the current study were procured from the same commercial supplier as the rats used by Floresco and colleagues (2009). Since the saline treated offspring reared in our facility performed similar to rats from commercial suppliers, it suggests that these offspring are comparable to rats raised by commercial suppliers. Thus, the results generated from this study

can be compared to other studies investigating similar hypotheses, given that the saline treated offspring do not differ from the commercially procured animals.

Prenatal treatment with PolyI:C did not disrupt the acquisition of the visual-cue discrimination. Both PolyI:C treated male and female rats were not significantly different from the saline treated offspring. This suggests prenatal PolyI:C administration does not affect simple discrimination learning. Furthermore, PolyI:C did not affect motivation to press levers for reward or induced motor impairments, as the average response latencies were similar between the PolyI:C and saline groups across all test days. However, female adult offspring took more time in responding to the lever when compared to the male offspring. This behavioural output is consistent with other data, which will be elaborated on in a subsequent section.

Effects of Acute PolyI:C Treatment on Dams and Pups

The increase in temperature after an acute injection of PolyI:C reflects an inflammatory response similar to the acute phase of a viral infection (Meyer, 2008a). Both increased temperature and decreased weight of the treated dams have been shown in other studies (Fortier et al., 2004; Zuckerman et al., 2003; Zuckerman & Weiner, 2005). Interestingly, the weight loss of the treated dams did not affect the litter size or weight of pups and again is in line with other studies (Schwendener et al., 2009; Zuckerman et al., 2003; Zuckerman & Weiner, 2005). Specifically, Zuckerman and Weiner (2005) found that 4mg/kg of PolyI:C, administered on GD 15, induced weight loss for one day in the Wistar rat without affecting litter size. As well, administration of 5mg/kg of PolyI:C on GD17 did not alter the weight of pups at birth in mice (Schwendener et al., 2009). From this, it is evident that the PolyI:C administered in the current experiment has replicated previous findings and therefore, demonstrates that the PolyI:C treatment worked.

The comparable litter size and pup weight between the treatment and control groups, despite the weight decrease in the treated dams, has important implications. For example, malnutrition during pregnancy has shown to induce serious alterations in fetal development (Meyer & Feldon, 2010). Meyer and Feldon's review (2010) noted that prenatal protein deprivation altered basal and stress induced dopamine (DA) and serotonin (5-HT) release in the prefrontal cortex and hippocampal regions. These structures are strongly linked to executive functions in humans (Brown et al., 2010; Fuster, 2008; Moritz et al., 2009; O'Brien et al., 2010) and animals (Bitanhirwe et al., 2010; Floresco & Ghods-Sharifi, 2006; Floresco et al., 2009; St. Onge & Floresco, 2010). Given that the litter size and weight of pups were similar between groups, it appears that malnutrition might not have played a significant role in altering the cognitive flexibilities seen in the male adult PolyI:C offspring.

Maternal Behaviour on Pups

Maternal behaviours are critical for the normal development of pups. The set-shifting impairment in the current study may be a result of maternal behaviour. Numerous studies have shown that decreases in some types of maternal behaviour (e.g., licking and grooming of pup or time spent on and off nest) induce behavioural and cognitive changes in the adult offspring (Barha et al., 2007; Lovic & Fleming, 2004; Meyer et al., 2008b; Schwendener et al., 2009; Zhang et al., 2005). Prenatal infection has been reported to produce changes in maternal behaviour (Meyer et al., 2008b; Schwendener et al., 2009). Schwendener and colleagues (2009) showed that maternal licking and grooming were reduced in the PolyI:C treated dams compared to saline treated dams. However, these results are inconsistent with the current data, given that differences in maternal behaviour were not observed between the two groups. Numerous factors could account for this inconsistency. For example, the sampling rate in the Schwendener and

colleagues (2009) paper was taken every 20 s per dam, for every 3 hrs, with a video recording machine. Recording sessions went into dark cycles (Schwendener et al., 2009). In contrast, the recording sessions for the current study were taken daily, during the light cycle, and had two 10 min samples per dam. Samples taken during the dark cycle may be a better measure to tease out the effects of maternal behaviour on cognitive and behavioural changes exhibited by the adult offspring. This is because nursing is the most active during the dark cycle (Swendener et al., 2009). As well, rats and mice may react differently to the PolyI:C treatment, as some differential effects have been shown in Meyer and Feldon's paper (2010). Therefore, maternal behaviour did not play a role in inducing set-shifting deficits in the treated male offspring.

Null Effect of PolyI:C Treatment in the Female Adult Offspring

PolyI:C treated female adult offspring performed similarly to the saline treated female offspring across all 3 test days. However, females took longer to respond to the lever than male rats. This data does not support the hypothesis that PolyI:C treated female offspring would be impaired during set-shifting and reversal learning. Nevertheless, the lack of cognitive impairment, more training days and slower lever response time are in line with other data (Longenecker et al., 2010; Mueller & Bale, 2007; Seidman et al., 1997). Sex differences in cognition are frequently seen in clinical settings. Male schizophrenic patients were shown to be more impaired in the WCST than female patients (Longenecker et al., 2010; Seidman et al., 1997). Male patients displayed great difficulty in suppressing the old reinforcing but currently non-reinforced strategy during the WCST (Seidman et al., 1997). Again, the increase in perseveration seen in male patients resembles the perseveration exhibited by the treated male offspring from the current experiment.

In animal studies, Mueller and Bale (2007) found that prenatal stress introduced at different gestation time points resulted in differential effects during the modified version of the Barnes maze. Specifically, they found that prenatally stressed (all time points) male mice offspring took more time, made more errors and made less successful entries in finding the target box than control male mice (Mueller & Bale, 2007). In contrast, female offspring that were prenatally stressed during the middle and late gestation performed similarly to the control female rats during this maze task. Interestingly, control female mice took significantly longer time to locate the escape box than control male mice during the training phase. This stress data is comparable to the current data since prenatal PolyI:C treatment can be viewed as a stressor (Meyer & Feldon, 2010). Acute PolyI:C treatment have shown to release IL-6, which is associated with stress responses (Meyer & Feldon, 2010).

In addition, age and dosage might have played a role in the null effect as well. Broberg and colleagues (2008) found that neonatal treatment with a NMDA antagonist impaired the adult male offspring's ability to set-shift during the EDS of the digging task. Interestingly, the same treated female adult offspring were also impaired on the EDS. In addition, only the treated females had a dose dependent effect, whereby the higher the dose of the NMDA antagonist, the more severe the impairment exhibited by the adult female offspring (Broberg et al., 2008). From this, it is reasonable to conclude that female rats are susceptible to EDS/set-shift deficits. The null effect of the PolyI:C female rats in the current experiment may be a result of an insufficient dose of PolyI:C to induce impairment. Most PolyI:C mice studies use 5mg/kg to mimic an infection (Meyer & Feldon, 2010). As well, the age that the adult offspring were tested in the current experiment is analogous to the human early adulthood (e.g., 18; Pantelis et al., 2009). It is known that the average age onset for the females is around 25 years old and males are around

18 years of age (Seidman et al., 1997). Furthermore, the study conducted by Broberg and colleagues (2008) tested female rats that were one month older than the male rats. Therefore, it could be that the PolyI:C female offspring were tested in an earlier time frame in which the potential cognitive deficits may not have manifested yet.

Most importantly; however, is the protective role of estrogen in the females (Barha & Galea, 2010; Behl, 2003). It has been well documented that estrogen increase both hippocampal neurogenesis and synaptic protein levels in the hippocampus (Barha & Galea, 2010). Hippocampus is an important region in facilitating learning and memory and it plays a critical role in the ability to set-shift, as neonatal lesion to the HPC impairs set-shifting (Brady, 2009). In addition, estrogen has been shown to directly modulate the hypothalamus and protein cascades, given estrogen receptors are found in the brain (Behl, 2003). Specifically relevant to the current experiment, estrogen directly decreases the activity of the glycogen synthase kinase 3 (GSK3; Behl, 2003). It has been shown that a decrease in GSK3 protein increase the release of the anti-inflammatory cytokines, whereas increased GSK3 levels releases pro-inflammatory cytokines (Martin et al., 2005; Woodgett & Ohashi, 2005). Given that acute PolyI:C injection significantly increase the levels of pro-inflammatory cytokines, it is reasonable to conclude that estrogen may have attenuated the pro-inflammatory response in the females, as opposed to the males, given that decrease in GSK3 increase anti-inflammatory cytokines (Martin et al., 2005; Woodgett & Ohashi, 2005). The balance between the anti and pro-inflammatory cytokines in the maternal environment have been shown to play a critical role in inducing cognitive and behavioral deficits in the adult offspring with prenatal infections (Meyer & Feldon 2010). Any increase in either direction (e.g., pro or anti-inflammatory) would induce cognitive deficits in the adult offspring (Meyer & Feldon, 2010). Given that the PolyI:C female adult offspring have

estrogen to decrease the levels of GSK3, by default, the PolyI:C females would be protected from the pro-inflammatory increase from the prenatal infection in the current experiment. Taken together, it is likely that the null effect of the PolyI:C treated female adult offspring seen in the current experiment can be explained by the potential neuroprotective effect of estrogen.

Future Directions

The strengths of this study are its face and construct validity. Face validity is the appearance in which a model is reflective of the symptoms or any other component of a human disease. Construct validity refers to the degree in which the animal model measures the underlying factors that may contribute to the disorder that is being examined. Both face and construct validity are supported, given that PolyI:C male offspring perseverated during the set-shifting day, while female PolyI:C offspring were not impaired. This sex difference is consistent with findings from the human literature, which examined patients with psychiatric disorders (Cannon et al., 1994; Fuster, 2008; Longenecker et al., 2009; Seidman et al., 1997; Titze et al., 2008) and with other prenatal infection studies (Bimonte et al., 2000; Bitanirwe et al., 2010a; Gresack & Frick, 2003; Meyer & Feldon, 2010; Zuckerman & Weiner, 2005).

However, there are some weaknesses in this study that can be resolved with additional experiments. For instance, the reversal learning facilitation exhibited by male PolyI:C adult offspring may not be a true facilitation but an artifact of being the second cue-dimension or strategic shift. If PolyI:C does in fact alter reversal learning, then this facilitation should also be seen during a serial reversal learning task. Testing these animals through such a reversal learning protocol would further elucidate how prenatal PolyI:C might impact this form of cognitive flexibility. Floresco and colleagues (unpublished data) found that subchronic treatment with ketamine, an NMDA antagonist, impaired the first reversal learning, during a serial reversal

learning protocol. Interestingly, treated rats showed facilitation on the 3rd reversal learning day, whereby these rats showed significantly fewer regressive errors. This data is consistent with OFC lesioned rats tested in serial reversal learning, whereby rats facilitated on the 3rd reversal learning day (Clarke et al., 2005). If prenatal PolyI:C exposure induces a similar pattern as the OFC lesion study, using a serial reversal learning task, then the reversal learning facilitation observed in the current study might not be a true effect.

Moreover, the order of the set-shift, from visual-cue to response discrimination, should also be counterbalanced. This would ensure that the set-shifting impairment exhibited by the male PolyI:C offspring is a robust effect. However, it is important to note that a set-shift from response to visual-cue discrimination have been shown to be an easier shift (Floresco et al., 2008). Thus, it may not be sensitive in detecting differences between the treated and saline groups.

As well, quantification of DA and 5-HT receptor subtypes should be examined in the mPFC and subcortical regions in the prenatal PolyI:C treated offspring. This would permit the examination of changes, if any, in cell density and if so, whether those changes correspond to the receptor subtypes that are known to be important mediators of set-shifting and reversal learning.

In addition, the current study examined only one form of cognitive function. More behavioural tests are needed to further validate that prenatal PolyI:C is a valid model of psychiatric disorders. Showing the set-shifting impairment is only the first step in trying to model psychiatric disorders. Other tests, such as the prepulse inhibition (PPI) may be used to examine whether prenatal treatment with PolyI:C would alter sensorimotor gating in the rat. Sensorimotor gating is typically impaired in schizophrenic patients (Fuster, 2008) and it is the ability to filter out irrelevant stimuli in the environment to attend to relevant stimuli. It has been

shown that PPI is dependent on the PFC; however, the exact mechanisms still needs to be elucidated (Meyer & Feldon, 2010). Wolff and Bilkey (2008) found that prenatal treatment with PolyI:C in the rat induced PPI impairments in both the adolescent and adult offspring. Other studies have also shown PPI deficits; however, the majority of the studies were conducted with mice (Meyer et al., 2010; Meyer & Feldon, 2010). In addition, the effects of prenatal PolyI:C treatment should be examined using the object in place task, as this task has been reported to be dependent on the prefrontal cortex (Warbuton & Brown, 2010). The object in place task initially allows the rat to explore 4 different objects in each corner of a square open field (Douma et al, 2011). After a delay, 2 of the 4 objects swap locations and the rat's memory of the spatial relationship between the objects is tested (Douma et al., 2011). If the rat remembers the spatial relationships between objects from the initial exposure, it will spend more time exploring the objects that changed position compared to the objects that remained in the same place (Douma et al., 2011). If treated adult offspring are impaired in this task, it would provide further support that prenatal PolyI:C treatment affects prefrontal functions.

Most importantly, predictive validity is currently missing from this study. Predictive validity refers to the pharmacological aspects of the study, whereby one examines whether the animal model show similar improvements after drug application. To further validate the relevance of the impairments induced by prenatal PolyI:C to schizophrenia, antipsychotics must be used. Some prenatal infection studies have shown that antipsychotics, such as clozapine, alleviated LI impairments and reversed the facilitation of reversal learning (Zuckerman & Weiner, 2005). Furthermore, Meyer et al, (2010) found that peri-adolescent treatment with haloperidol, clozapine or fluoxetine alleviated behavioural disturbances that were induced by prenatal PolyI:C treatment. Specifically, both fluoxetine and clozapine ameliorated PPI deficits

exhibited by the PolyI:C offspring. Additionally, haloperidol and clozapine alleviated LI impairments during an active avoidance task.

However, due to the lack of current antipsychotics for treating complex cognitive inflexibilities (Rodefer et al., 2009), such as set-shifting and reversal learning, novel components targeting cognitive deficits should also be examined. Currently, modafinil and SB271046 have been gaining therapeutic support in alleviating cognitive deficits in psychiatric disorders. One clinical trial test, using modafinil, showed a significant improvement in overall function and better performance in a subset of the Wechsler Adult Intelligence Scale for schizophrenic patients. More importantly, modafinil improved attentional set-shifting during a 3-dimensional shift task that had a WCST component and this improvement allowed the patients to live independently (Morein-Zamir et al., 2007). In addition, a study using subchronic NMDA antagonist treatment in the rodent showed that acute modafinil administration significantly decreased the number of trials to reach criterion during the EDS, in the digging bowl task (Goetghebeur & Dias, 2009). Furthermore, a subchronic NMDA antagonist study found that SB271046 alleviated set-shifting impairment in the same digging bowl task (Rodefer et al., 2008). SB271046 is a 5-HT₆ antagonist and has been found to increase levels of glutamate in the PFC (Rodefer et al., 2008). Given these results, it is important to test these substances using the current model to examine whether the cognitive alterations can be ameliorated. If so, these results would further validate the prenatal infection as a credible animal model for psychiatric disorders.

Conclusion

The data from the current study support the hypothesis that prenatal infection is an important factor in inducing long term changes in the fetal brain, which in turn increases the risk

of the offspring developing a psychiatric disorder, such as schizophrenia. Prenatal treatment with PolyI:C, impaired the ability of the male offspring to set-shift from a visual-cue to an egocentric response strategy. This deficit is consistent with the WCST impairments exhibited by schizophrenic patients (Longenecker et al., 2009; Seidman et al., 1997). In addition, facilitation of reversal learning was observed in the same male rats, whereby the number of regressive errors made during reversal day was significantly decreased. This facilitation was reported in another prenatal PolyI:C study (Zuckerman & Weiner, 2005); however, it is inconsistent with clinical studies (Leeson et al., 2009; Pantelis et al., 1999), as well as another prenatal animal study (Meyer et al. 2006a). This discrepancy can only be resolved with further examination of the role of prenatal infection in altering reversal learning. Lastly, PolyI:C female offspring were not impaired during any of the test days. However, all females were slower in responding to the lever than male rats. Again, the sex difference is reminiscent of clinical studies, in that male schizophrenic patients were much more impaired during WCST than female schizophrenic patients (Longenecker et al., 2009). Furthermore, the symptoms exhibited by female schizophrenic patients are less severe than male patients (Pregelj, 2009). Taken together, the results from the current study have both face and construct validity. However, further studies are needed to resolve the reversal learning component of this prenatal model. Ultimately, prenatal PolyI:C treatment may be a viable model to test for new therapeutics that may prevent or treat psychiatric disorders, such as schizophrenia.

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ANIMAL USE PROTOCOL #
20080107

TITLE
Maternal infection as a preclinical model for behavioural and electrophysiological alterations associated with schizophrenia

APPROVAL DATE:
October 19, 2009

APPROVAL OF:
New / Renewal Animal Use Protocol

EXPIRY DATE:
October 19, 2010

Full Board Meeting AREB Subcommittee AREB Chair and University Veterinarian AREB Chair

CERTIFICATION

The University of Saskatchewan Animal Research Ethics Board reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period.

PROTOCOL MODIFICATIONS

Any modifications to this protocol must be approved by the UCACS AREB Chair prior to implementation, using the [AUP Modification Form](#).

ONGOING REVIEW REQUIREMENTS

Research programs that extend beyond one year must receive annual review. For the annual renewal, an annual review form (and progress report) must be submitted to the AREB within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the [Research Ethics Office website](#) for further instructions.

Jane Alcorn, DVM, PhD, Chair
Animal Research Ethics Board
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November 4, 2009
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