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An Investigation of Short-Term Memory Functioning in a Neurodevelopmental Rat Model of Schizophrenia

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An Investigation of Short-term Memory Functioning in a Neurodevelopmental Rat Model of Schizophrenia

by

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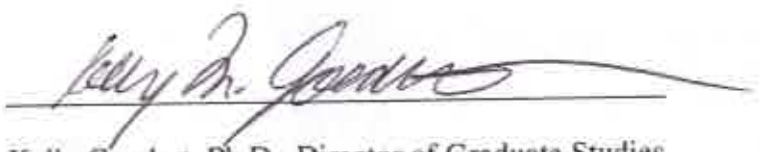

Kelly Goedert, Ph.D., Director of Graduate Studies

Dedication page

To my wonderful parents, my sister and brother, and my grandparents, who always took the time out to listen to my rat stories.

To my loving boyfriend and family who always made it possible for me to breathe.

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Abstract

Schizophrenia is a debilitating disorder characterized by positive, negative, and cognitive symptoms. Cognitive deficits affect one's learning and memory due to a dysfunction in the brain, inhibiting a normal functioning life. The self-medicating hypothesis states that ninety percent of people with schizophrenia smoke, because nicotine use improves these cognitive deficits. The cytokine hypothesis states that inflammation due to prenatal infection is associated with schizophrenia. These increased proinflamatory cytokines can damage the brains of the fetuses, which can cause schizophrenic-like symptoms in rats. The present study used Lipopolysaccharides to activate the cytokine-mediated inflammatory response injected into pregnant dams. A T-maze was used to measure short-term memory in rats. The goal of the present study was to test if rats with LPS-treated mothers had cognitive deficits mimicking schizophrenic-like symptoms, and if so, if nicotine could improve those cognitive deficits. The present study consisted of spontaneous alternation, alternation training, delayed alternation training, and nicotine treatment. The hypothesis could not be adequately tested in the present study because there was a small sample size of LPS-treated animals as a result of high rate of pregnancy loss. The LPS-treated animals did spontaneously alternate, but did not learn the other instrumental tasks and could therefore not be tested for short-term memory deficits. Short-term memory was utilized in the healthy control pups. The latencies across the alternation training days decreased across healthy control rats, they were successfully alternating, and performed above chance, indicating use of their short-term memory. On most tasks females performed significantly better than males did. Nicotine did not significantly improve performance, but it continued to stay above chance.

An Investigation of Short-Term Memory Functioning in a Neurodevelopmental Rat Model of Schizophrenia

Schizophrenia is a human mental disorder that impairs thought processes and emotional responses (U.S. Department of Health and Human Services, 2009). One percent of the world's human population is affected, and these patients tend to suffer with this disorder for their entire lifetime (Orellana & Slachevsky, 2013). Schizophrenia has been researched for over 100 years with no successful cure (Rossler, Salize, Van Os, & Riecher-Rossler, 2005). Although research has come farther with treatments than when the illness was first described in 1896, there are many who never recover (Rossler et al., 2005). Suicide is the cause of death for 10% of patients (Orellana & Slachevsky, 2013) making the patient lifespan about 10 years younger than the average person (Rossler et al., 2005). Not only is this illness debilitating for the patient, it also takes a major toll on their family members (Rossler et al., 2005). In 2002, the burdensome disorder schizophrenia cost $62.7 billion (Wu, Birnbaum, Shi, Ball, Kessler, Moulis, & Aggarwal, 2005). The large unemployment rate in schizophrenics makes up some of most of this cost, but also include costs for health care and family support (Wu et al., 2005). It is costly to maintain a healthy living style for these patients and their families. More research is needed to understand how to prevent this illness and to treat it as soon as it arises. This will lower costs and improve the lives of these patients reintegrating into society.

Characteristics of Schizophrenia

Positive and Negative Symptoms. According to the U.S. Department of Health and Human Services (HHS, 2009), there are three categories of schizophrenia: positive, negative, and cognitive symptoms. Positive symptoms consist of hallucinations, disorders of thought and movement, and delusions (HHS, 2009). Positive symptoms are linked to dopamine transmission

and can be improved by neuroleptic drugs (Angrist, Rotrosen, & Gershon, 1980). The negative symptoms are abnormal emotions and behaviors, for example, flat affect which is when patients' faces are expressionless and they are monotonous when they talk, not speaking much, and not finding pleasure in life (HHS, 2009). Negative symptoms have been thought to be linked to the structure of the brain and are harder to improve than positive symptoms (Angrist et al., 1980). Additionally, executive functioning is a very prominent deficit in people with Schizophrenia (Orellana & Slachevsky, 2013). Most deficits in executive functioning fall under the category of negative symptoms. Executive functioning is highly linked to working memory. It includes impairments in problem solving, planning, cognition, and verbal skills. It is thought that the prefrontal cortex (responsible for executive function) does not function properly in patients with schizophrenia (Orellana & Slachevsky, 2013).

Cognitive deficits as a core component in Schizophrenia. Cognitive symptoms were originally thought to be impaired only in elderly patients with Schizophrenia, but it is now known that cognitive impairments normally occur before the onset of Schizophrenia (O'Carroll, 2000). These symptoms occur in 75% of Schizophrenics affecting their memory, attention, motor skills, executive functions, and intelligence (O'Carroll, 2000). There have been laboratory-based studies showing cognitive improvements but nothing to improve Schizophrenic's daily life functioning on a long-term basis (O'Carroll, 2000). Evidence from O'Carroll (2000) suggests that most of the impairment comes from a dysfunction of the frontal and temporal lobe, affecting how memory functions as well as executive functioning. Some examples of these memory problems are seen when patients have difficulty distinguishing between errors and correct responses (O'Carroll, 2000).

The cognitive symptoms are subtle, such as poor executive functioning, problems with working memory, and trouble focusing, and are usually only detected when sensitive tests are performed (HHS, 2009). They also have emotional withdrawal and perceptual abnormalities (Malhotra, Pinals, Adler, Elman, Clifton, Pickar, & Breier, 1997). Tasks are done in animal models to test problems with working memory and executive functioning mimicking the deficits of humans (Malhotra et al., 1997). Therefore, with an impaired working memory, it is difficult to store information into long term memory, inhibiting people from being able to function (Aleman, Hijman, de Haan, & Kahn, 1999). These disturbances in basic cognitive functions make it hardest to lead a normal life (HHS, 2009).

Self-medication hypothesis and smoking in schizophrenia. Ninety percent of Schizophrenics smoke cigarettes (Levin, Wilson, & McEvoy, 1996). The self-medicating hypothesis explains this high prevalence of smoking in Schizophrenics by saying that nicotine medicates Schizophrenic symptoms (Dalack, Healy, & Meador-Woodruff, 1998). This directly relates to the cognitive symptoms of Schizophrenia because nicotine may help reduce cognitive deficits in attention and short-term memory (Newhouse, Kellar, Aisen, White, Wesnes, Coderre, ...& Levin, 2012). Kumari and Postma (2005), for example, have measured prepulse inhibition (PPI) in Schizophrenics, a task commonly used in animals and humans to examine the disruptive effects of a weaker distracting stimulus (a pre-pulse) on a startle reflexive response to a subsequent stronger stimulus. Typically, people and animals show a reduced startle response on trials with a pre-pulse compared to trials without a prepulse. This reduced response is called pre-pulse inhibition. People with Schizophrenia have deficits with this PPI showing a weaker PPI effect. PPI is believed to reflect a normal pre-attentive process that serves to help regulate attentional processes. When the pre-pulse is presented, attention is directed at this initial stimulus

and attention to other stimuli is reduced to reduce cognitive overload. PPI deficits in Schizophrenia suggest that these individuals are unable to effectively "tune out" stimuli, which leads to overstimulation and makes them unable to avoid interference, creating behavioral confusion. If nicotine can reduce attentional deficits, then smoking might be a self-medicating process for these schizophrenic symptoms (Kumari & Postma, 2005).

Research also suggests that nicotine enhances working memory in people with schizophrenia (Carlson, Gilbert, D. G., Rise, H., Rabinovich, N. E., Sugai, C., & Froeliger, 2009). In an experiment by Levin, Wilson, and McEvoy (1996) Schizophrenics were impaired in their performance on a short-term memory task after overnight abstinence from smoking, but participants who received nicotine performed better on the test. Improved cognition is believed to be due to nicotine binding to acetylcholine receptors in the brain (Xiu, Puskar, Shanata, Lester, & Dougherty, 2009), specifically in the dorsolateral prefrontal cortex (Yang, Paspalas, Picciotto, Arnsten, & Wang, 2013). The first study testing if PPI in humans was affected by nicotine was done in 2006 (Postma, Gray, Sharma, Geyer, Mehrotra, Das, ...& Kumari, 2006). It was found that nicotine also enhances PPI in healthy controls. This can be tested by looking at the limbic activity (Postma et al., 2006). This suggests that with more research it may be found that nicotine enhances other aspects of healthy and schizophrenic brains more so than just PPI. The studies reviewed, demonstrate deficits in attentional and working memory in schizophrenics. The evidence supporting the hypothesis that Schizophrenics may smoke to reduce these symptoms is intriguing, and supports the contribution of neurocognitive dysfunction in schizophrenia, but requires much more research to evaluate.

The Need for Animal Models

Rat models cannot model the complete disorder of schizophrenia due to the fact that psychiatric disorders are very complex. However, it is important to study mental disorders in animal models because animals can be manipulated to trace potential causes and investigate treatments of disorders. Animal models can be more closely studied to examine specific parts of a disorder, such as cognitive symptoms of Schizophrenia to help further research and treatment for humans. There are three types of validity used in research today to evaluate if animal models accurately represent human diseases. Dodd (1999) states that *face validity* is how well the model represents the identifying features of the disease, *predictive validity* is if drugs can reduce symptoms in animals, as they can help to be reduced in humans with the disease, and *construct validity* is if the model includes a core causal factor of the disease. A model with strong construct validity uses a cause of the disease that is well known. Unfortunately, the causes of most neurological diseases in humans, including Schizophrenia, are not known. Chadman, Yang, and Crawley (2009) stated that causes of human diseases could be more accurately modeled if animals were exposed to, lesioned, or prenatally given toxins. Genes are suspected causal agents in many neurological disorders as well.

Chadman et al., (2009) said that a good way to test a hypothesis of a particular gene being causally related to Schizophrenia, would be by inhibiting the gene (e.g. the COMT gene) in an animal model. Nestler and Hyman (2010) stated that one might be able to achieve good construct validity in animals by inserting alleles associated with a human disease. Looking at the DISC 1 gene, for example (a gene associated with Schizophrenia in humans that regulates cell proliferation, migration and differentiation during development) could help gain insight into Schizophrenia in humans since it is a gene also in animals (Chadman et al., 2009). Alleles

though, are not always compatible enough to provide the same neuronal death that is seen in human neuropsychiatric disorders (Nestler & Hyman, 2010). Another problem with alleles as causal agents is that a specific gene can cause different symptoms in different people. For example, a mutation in the DISC 1 gene is associated with Schizophrenia as well as bipolar disorder (Nestler & Hyman, 2010). Therefore, the causal influence of this gene may depend on other biological and environmental factors. Alleles modeling a human disease may have different effects on a rat, meaning accurate construct validity for a model of Schizophrenia would not be attained. Even when similar causal effects have been observed in animals, the effect size in previous research has not been large enough to increase confidence in the construct validity of the animal model (Nestler & Hyman, 2010). Evidently, it is difficult to assess whether or not an animal model of Schizophrenia has accurately attained all three types of validity.

If it is not yet possible to create animal models with known causative factors of Schizophrenia (models with good construct validity), the alternative strategy is to attempt to achieve better face validity by modeling the symptoms of the disease. Many DSM criteria explaining these symptoms cannot be diagnosed in animals though (Nestler & Hyman, 2010). However, Nestler and Hyman (2010) argue that the cognitive symptoms can be replicated in animal models most prominently. Chadman et al., (2009) say that endophenotypes can be modeled in animals. Endophenotypes are specific markers for a disorder such as specific symptoms of behavior and neurophysiological responses (Chadman et al., 2009). Animal models with good face validity seem to be the most appropriate rodent model of Schizophrenia at this time. Therefore, the goal of the present research was to model a cognitive symptom of Schizophrenia and, as will be explained below, to test exposure to maternal immune system

activation as a potential cause of disturbed cognition. The successful finding that exposure to maternal immune activation disturbs cognition in the rat will confirm that disturbances in cognition can be demonstrated in rats (face validity) and provide some support for the hypothesis that immune system activation plays a causative role of cognitive deficits in Schizophrenia (Chadman et al., 2009).

Subsequently, this study will attempt to model cognitive "endophenotypes" that are relevant to Schizophrenia (Boksa, 2007). The term endophenotype was introduced by psychiatric geneticists to suggest a strategy for identifying the genetic mediation of complex psychiatric disorders. Rather than look for all of the genes associated with the phenotype of a psychiatric disorder, it may be easier to identify the genes for specific components or traits making up the disorder. A presumed component or trait of the phenotype of a psychiatric disorder is an endophenotype. Boksa (2007) suggested applying this concept of endophenotype to animal models of human psychiatric disorders. In an animal model of Schizophrenia one or more endophenotypes can be identified and experimental manipulations introduced to test hypotheses concerning the modulation of these endophenotypes.

The endophenotype to be studied in the proposed experiment is psychological/behavioral function, such as working memory and other cognitive factors, which are typically observed to be disordered in Schizophrenia. Evidence suggests that vulnerability to Schizophrenia may be increased by maternal bacterial and viral infections that expose the developing fetus to high levels of inflammatory cytokines (HHS, 2009). When fetuses are exposed to maternal infection, it affects their neurodevelopment, which increases their genetic vulnerability to Schizophrenia (Khandaker, Zimbron, Lewis, & Jones, 2013). A good rat model could help test the hypotheses

that exposure to elevated cytokines during development may result in altered cognitive function that is typically seen in Schizophrenia (Kumari & Postma, 2005).

An animal model that successfully results in an endophenotype of Schizophrenia through prenatal infection exposure can then serve as a model to test additional hypothesis about Schizophrenia (Kumari & Postma, 2005). An example of this would be the self-medication hypothesis, which has been proposed as an explanation of increased nicotine use (smoking) in Schizophrenia (Kumari & Postma, 2005). This model relates to what would be learned about cognitive symptoms from this project because the rats may be able to associate this self-medicating hypothesis with improving their cognitive symptoms. This means that the rats would realize the nicotine is improving their cognitive deficits and would voluntarily take the nicotine to improve the deficits.

Measures of cognition in rats (long-term and short-term memory). Rat brains share important "design principles" with humans such as the connectivity between the cortex and sub-cortical areas (Abbott, 2010). Several tasks have been developed to measure presumed cognitive performance in rodents. The Morris water maze was introduced in the early 80's by Richard Morris in Scotland, and is used to study learning and memory at the neuronal level. Rats swim in a water filled pool and must find a hidden platform to escape. With repeated training the rats find the escape platform quicker by relying on their memory of the platform location (Morris, 2008). Studies with the water maze demonstrate that rats rely on spatial memory when extramaze cues are available, but may use several other search strategies that do not require the need to access a memory for the spatial location of the escape platform. For this reason, simply testing a rat in a water maze does not guarantee that the rat is using a memory strategy that is of interest to the experimenter (e.g., exclusive reliance on the long term memory of the platform

location) (Morris, 2008). Nevertheless, variations in the water maze task are very commonly used to assess "cognitive" function in rodents such as long-term memory and short-term or working memory (Morris, 2008). Working memory involves the attentional and executive control of short-term memory while STM is the temporary holding of relevant information during a task. However, many animal researchers use the term short-term memory and working memory as synonyms. In this thesis, the term short-term memory is preferred over working memory when discussing animal studies because direct evidence of attentional and executive control of temporarily held information is not available in these experimental procedures.

The eight-arm radial maze was designed in 1976 by Olton and Samuelson before the water maze was introduced, and is another widely used maze used to assess spatial memory in rats, particularly STM (Dubreuil, Tixier, Dutrieux, & Edeline, 2003). The rats are tested to find food in each arm. An effective strategy is to retrieve the food from each arm without revisiting an arm that was previously visited. By remembering which arm was previously visited the animal is relying on STM to achieve an effective search strategy. The radial arm maze has also proved similar results with other species. Roitblat, Tham, and Golub, (1982) used the radial arm maze with fish and found that when given freedom to choose an arm, the fish tended to move through the arms in a stereotypic direction. Search strategies to find food efficiently in the radial maze without relying on STM has also been observed in rats. Dubreuil et al., (2003) reported that rats can be influenced to rely on memory of the arms that have already been visited by enclosing the animals in each arm for approximately 10-15 seconds. Therefore, although the water maze task and radial maze task are very popular to study long-term and short-term memory in rodents, identifying precisely which strategies rats are using in these tasks can be tricky and may require considerable experimentation and testing. One cannot simply assume that

rats tested in these tasks are always utilizing the same cognitive strategies (even when commercially available apparatuses are used).

A T-maze is a much simpler apparatus that has also been used to examine cognitive and motivational processes in rats (Gaffan & Davies, 1982). Some T-mazes are designed where one arm of the T leads to an exit and the other arm leads to an enclosed space. When trials are done without much time in between (about 60 seconds), rodents tend to try the arm not visited previously. This behavior suggests that the rodents remembered their first choice and therefore have chosen the alternate pathway. This behavior in the T-maze is known as *spontaneous alternation* and is assumed to depend on short-term memory (Gaffan & Davies, 1982).

There is also the *reward alternation* or *delayed-alternation* procedure where an animal is deprived of food and then rewarded if they alternate sides across trials, with the time (or delay) between trials manipulated by the experimenter. Marquis, Goulet, and Dore, (2008), for example, found short-term memory deficits following neonatal hippocampal lesions in 23 day-old rats tested in a delayed alternation task with various delays. In a separate experiment, the hippocampally-lesioned rats were tested for spontaneous alternation, where they investigated the T-maze without the guillotine doors for 15 minutes. The rats determine the delay between the arm choices made rather than the experimenter since they are freely exploring the maze. When the rats took less than 5 seconds between arm choices, both the lesioned and control rats showed consistent alternation. However, when the rats took greater than 5 seconds between arm choices, spontaneous alternation continued in the control animals, but the lesioned animals no longer showed spontaneous alternation behavior. This result indicates that rats with neonatal hippocampal lesions experience a deficit in working/short-term memory that disrupts performance when a task requires memory across longer delays (delayed alternation task) and

when the rats delay exploration of the next arm in a maze for more than 5 seconds on their own (Marquis et al., 2008).

The T-maze has the least amount of limitations and is the best choice for investigating STM in the present study. There are only two ways to go as opposed to the eight-arm radial maze, and it is not an aversive task as is the water maze task. Rats fear the water and therefore it introduces an added motivation that could impact on cognitive performance.

Etiology of Schizophrenia

Although the cause of schizophrenia is unknown, evidence suggests that genes and the environment contribute to disturbances in brain chemistry and brain structures that are associated with schizophrenia (Borrell, Arevalo, Molina, & Guaza, 2002).

Genes. Abnormal connectivity between the hippocampus and the temporal lobes appears to be an important part of the problems of Schizophrenia (Sigurdsson, Stark, Larayiorgou, Gogos, & Gordon, 2010). To study this neural circuitry, Sigurdsson et al. (2010) developed a genetic mouse model of Schizophrenia because the pathophysiology of this disease can be better understood by looking at the genetic risk factors. Evidence suggests that there is a genetic link between the human chromosome 22 and Schizophrenia (Polymeropoulos, Coon, Byerley, Gershon, Goldin, Crow, & Delisi, 1994). Sigurdsson et al. (2010) studied mice, which modeled the deletion of a small region of this chromosome 22. Neural activity between the hippocampus and the prefrontal cortex was measured while the rats were doing a working memory task. The authors found a decrease in how synchronized the neurons were between the frontal lobe and hippocampus that is typically seen during the working memory task. These results suggest that electrical communication at the level of the neuron may be able to explain functional

connectivity problems leading to deficits in cognitive functioning and therefore working memory. This study only demonstrates one aspect of the cognitive symptoms of Schizophrenia (Sigurdsson et al., 2010).

Kvajo, McKellar, Arguello, Drew, Moore, MacDermott, and Gogos, (2008) conducted an experiment using mice with a mutation of the Disc 1 gene. The protein produced by the Disc 1 gene interacts with other proteins that are critical for cortical development (Kyajo et al., 2008). The Disc 1 gene (Disrupted-in-schizophrenia 1) is expressed in the hippocampus and is known to be a candidate for schizophrenia (Callicott, Straub, Pezawas, Egan, Mattay, Hariri &Weinberger, 2005). Callicott et al. (2005) altered the alleles in this Disc 1 gene where the alterations were visible in the hippocampus and increasing the risk for Schizophrenia. Kvajo et al., (2008) demonstrated that mice genetically engineered to model the human Disc 1 mutation show altered mature and newly formed neurons of the dentate gyrus. The results of this study also showed a deficit in short-term memory performance, which the authors tested for with the Morris water maze and a radial arm maze. Kyajo et al. (2008) said the memory deficit was due to a failure of neural circuits in the hippocampus, which supports the hypothesis that Disc1 is a vulnerability gene for Schizophrenia.

Stress as an environmental factor during neurodevelopment. Stress can be an environmental factor that affects symptoms or onset of Schizophrenia. The diathesis-stress model states that there is a genetic predisposition that can cause one to react more intensely to stressful situations. These stressors can make Schizophrenic symptoms worse, but do not cause the symptoms (Read, Perry, Moscowitz, & Connolly, 2001). Bitanihirwe and Woo (2011) discuss how oxidative stress, meaning damage to proteins and lipids, can increase the rate at which Schizophrenia progresses. Pregnant women exposed to the stressful event of the invasion

in the Netherlands had a higher rate of children developing Schizophrenia (van Os & Selten, 1998). This shows that stress can have a significant impact on development of Schizophrenia prenatally, due to a genetic predisposition for it, and due to its damage to cell function.

Neuroinflammation from bacterial/viral infection (The Cytokine hypothesis).

Previous research provides evidence that there is an association between prenatal exposure to infection and Schizophrenia (Borrell et al., 2002). The risk of mental illnesses can be increased by environmental events in the form of viral and bacterial infections when fetuses are exposed prenatally (Ratnayake, Quinn, Walker, & Dickinson, 2013). Alterations in the immune system during maternal infection may result in abnormal fetal neurodevelopment by activating maternal proinflamatory cytokines (Ratnayake et al., 2013). Cytokines are modulators of the inflammatory response of the immune response to infection and also serve as regulators for the brain's development (Watanabe, Someya, & Nawa, 2010). Although maternal bacteria and viruses do not cross the placenta to directly infect the fetus, the maternal cytokines that are activated by the maternal immune system do cross the placenta to impact the fetus (Watanabe et al., 2010). This is because the cytokine levels are affected and become inflamed due to the maternal exposure to the infection (Urakubo, Jarskog, Lieberman, & Gilmore, 2001). Therefore, maternal infection may result in the excessive recruitments of cytokines to create pathological inflammatory processes in the developing fetus. This hypothesis, known as the *cytokine hypothesis,* explains that it is the increased presence of the proinflammatory cytokines that is causing the damage rather than the bacteria or virus itself (Watanabe et al., 2010).

Modeling the Inflammatory Response from Bacterial Infection in Rats

An animal model of Schizophrenia that is based on the cytokine hypothesis would require activation of the maternal inflammatory response. Lipopolysaccharides (LPS), large molecules on bacteria, are commonly used instead of live bacteria or a virus to activate a cytokine-mediated inflammatory response because it produces a model consistent with the cytokine hypothesis as well as one with good construct validity of a presumed cause of Schizophrenia (Borrell et al., 2002).

Ratnayake et al. (2013) found results supporting that after the mother rat is treated with LPS, cytokines are altered in the fetal brain, however further research is needed to see if cytokines are a primary factor that lead to mental illnesses. LPS also increases cytokine production in human monocytes and macrophages (Rossol, Heine, Meusch, Quandt, Klein, Sweet, & Hauschildt, 2011). Maternal LPS treatment has been shown to alter gene expression in the fetal brain (Oskvig, Elkahloun, Johnson, Phillips, & Herkenham, 2012). Urakubo et al. (2000) injected LPS into pregnant rats resulting in elevation of the placenta interleukin, an increase of tumor necrosis in amniotic fluid, and a decrease of tumor necrosis in the fetal brain. Among the genes that were abnormally expressed were genes that control general development as well as genes associated specifically with brain development (Oskvig et. al, 2012). Therefore based on this previous research this present study will aim to activate the immune system of pregnant rats with LPS. It is hypothesized that maternal LPS treatment will alter the fetal environment therefore, altering the expression of neurodevelopmental genes and brain development and giving the offspring cognitive Schizophrenic-like symptoms (Oskvig et al., 2012).

Previous research has shown that early attentional mechanisms of rats born from mothers treated with LPS while pregnant are decreased when later tested as adults (Borrell et al., 2002). These attentional mechanisms consist of cognitive functions allowing a person to attend, think, reason, remember, and comprehend ideas (HHS, 2009). Therefore, when attentional mechanisms are disrupted early in development they may cause impairments leading to an increased risk of Schizophrenia. In the proposed study however, the focus will not be on early attentional mechanisms, but on the memory component of cognition. Rats remember past moments and remember to change their actions for future tasks (Crystal, 2012).

Goal of the Present Experiment

The goal of the present experiment is to examine if maternal immune system activation affects STM performance in the offspring. Based on previous research's success and questions they allude to, this present study will include STM performance of rats born to mothers treated with LPS during pregnancy with rats born from saline-treated controls. A T-Maze will be used to measure spontaneous alternation and delayed spontaneous alternation (i.e., rewarded alternation). Spontaneous alternation is a task where the rats have 15 minutes to explore the maze and it is calculated how many times they enter an arm. Delayed alternation is when the rats will have 30 seconds and then 60 seconds in between trials and they must enter the arm that was not previously entered before. The hypothesis is that there will be a difference between groups in performance of spontaneous alternation because the rats from LPS-treated mothers should have poorer short-term/working memory and therefore show fewer alternations in the T-maze when exploring the maze initially (spontaneous alternation) and when later trained to alternate for food reward (delayed alternation). Nicotine absence has been shown to impair

working memory, and nicotine treatment has been shown to enhance working memory (Carlson et al., 2009). Nicotine also activates circuits in the brain to make you more awake and active (Yang et al., 2013). Therefore, the present study will then look to see if working memory increases to create more alternations in rats after nicotine treatment.

Part 1 of testing the rat model is spontaneous alternation. Spontaneous alternation in rats is driven by a natural bias to visit new locations during exploration that is dependent on, and the ability to maintain a short-term memory for the most recently visited locations. An advantage of the spontaneous alternation procedure is that it is easy to examine if an experimental manipulation (such as maternal immune system activation) causes changes in a natural behavior that is dependent on STM. Disadvantages of this procedure are that the numbers of tests of spontaneous alternation are limited because the exploratory behavior habituates. This habituation occurs if there is no motivation to continue to explore the T-maze. Another disadvantage is that STM is under the animal's control and therefore, the demands on STM cannot be experimentally manipulated by the experimenter.

Part 2 of testing the rat model is forced alternation training. Forced alternation training solves the problem of lack of motivation to explore in the rats, since food-deprived rats are motivated to alternate across trials to receive food reward. With consistent alternation behavior, many days/weeks of training are possible to test STM effects. The experimenter can directly manipulate STM by imposing different delays between visits to the left and right goal boxes. Differences between LPS-treated and saline-treated can be easily investigated. Manipulations known to improve STM performance can be tested. For example, nicotine should improve performance on forced alternation training at one or more delay intervals.

If a significant difference between groups is observed in the delayed alternation task, it can be assumed that the rats consistently demonstrate a known symptom of Schizophrenia, and a deficit in STM. The rats will be injected with a dose of nicotine to determine if the deficit can be reduced as predicted by the self-medication hypothesis. If no group differences are observed in the initial delayed alternation task, the delay between trials will be systematically manipulated to better characterize the effect of the delay interval on performance. Cognitive deficits are also a result of other mental disorders, and since research suggests nicotine improves cognition, it may be that this model can be attributed/generalized to other disorders apart from Schizophrenia.

Method

Subjects

Four experienced female breeder rats and two experienced male breeder rats were used to produce offspring to serve as the subjects for this study. They were on a normal food and water diet with 12 hours of light and 12 hours of dark conditions. The female breeder rats were injected with Saline or LPS diluted in saline solution at 15 gestational days of pregnancy. Two litters were born to saline treated rats with litter sizes of 11 (7 males, 4 females) and 12 (8 males, 4 females). Two litters were born to LPS-treated rats with litter sizes of 1 (male) and 2 (females). The rats were housed with their mothers until weaning on Day 21, and then rats were housed in same-sex quads or doubles in standard shoe-box cages. Approval of the Seton Hall Institutional Animal Care and Use Committee was obtained before the start of any experimental procedures.

Apparatus

A T-Maze was used to test if the rats demonstrated cognitive deficits. It was constructed out of wood using published specifications for a standard T-maze (Deacon & Rawlins, 2006) and

is shown in Figure 1. The start alley was 50 x 16 cm, and the two goal arms were 50 x 10 cm. Guillotine doors were placed at the entrance of each goal arm, but the central partition and guillotine door in the start arm were omitted.

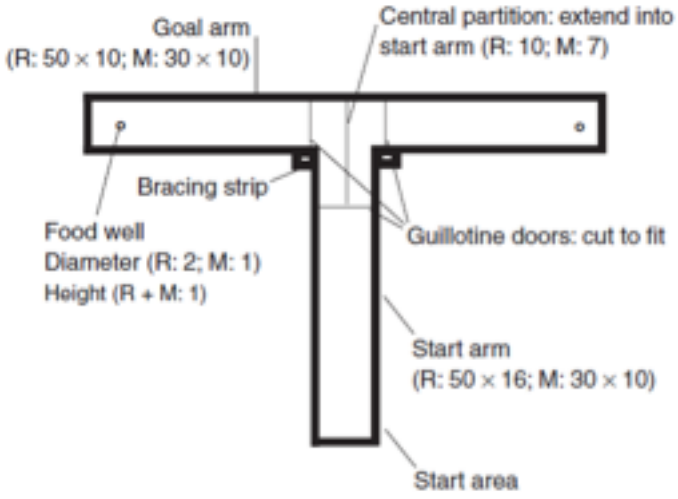

Figure 1 | T-maze plan. Dimensions are in cm: R = rat, M = mouse. For enclosed mazes, walls should be 20 cm high (mouse), 30+ cm (rat); for elevated mazes, 1 cm (mouse), 3 cm (rat).

Figure 1. T-Maze structure from Deacon & Rawlins (2006)

Drugs

The lipopolysaccharides (LPS) and nicotine were purchased from Fisher Scientific. LPS was dissolved in saline and administered at doses ranging from 0.0625 to 0.5 mg/kg (ip). Nicotine treatment was introduced after they rats completed delayed alternation training (see procedure). Nicotine was administer as 0.5 mg/ml/kg and prepared on the day of treatment by dissolving the drug in saline.

Procedure

LPS treatment. The two female rats were initially mated with the two male rats and

checked daily for a vaginal plug indicating a successful mating. On day 15 of gestation two of the experimental mothers were injected with 0.5 mg of lipopolysaccharide saline solution intraperitonally. After the two pregnancy losses at the 0.5 mg dose, the same two experimental mothers were injected, one with 0.25mg/kg, and the other at 0.125mg/kg of LPS at approximately 15 gestational days of pregnancy. The 0.25mg/kg induced another pregnancy loss, but there was one lone survivor pup of the 0.125mg/kg of LPS. The control mothers were injected with the saline solution vehicle. Both of the control mothers were impregnated again and became experimental mothers, receiving LPS, one at 0.125mg/kg, and the other at 0.0625mg/kg at approximately 15 gestational days of pregnancy. The 0.125mg/kg induced another pregnancy loss, but two LPS-induced offspring were born at 0.0625mg/kg. The rat pups were weaned at 21 days and periodically several 45 mg sugar pellets (Noyes Co.) were scattered in the cages to familiarize the rats with the reward for later training and testing. Testing started at 30 days of age with a total of 26 rats, 23 Healthy Controls offspring (HC) and 3 LPS-treated offspring.

Spontaneous alternation test. All rat offspring were tested in a spontaneous alternation task as described by Marquis et al (2008). This test allowed the rats to adapt to the apparatus while at the same time collecting data for evidence of group differences in spontaneous alternation. In the spontaneous alternation test the rats chose which arm they visit while exploring the T-maze without the guillotine doors blocking entry into the arms. The rat was placed in the start area of the start arm and was permitted to explore for 15 minutes. The sequence of entries into the 2 goal arms and the start arm was recorded for the full 15-minute duration. Entry into an arm was defined as when the rat's four paws were in an arm. The sequences of entries were scored for the number of spontaneous alternation episodes. A

spontaneous alternation episode was defined as when the rat visited the three different arms in succession. Following the scoring method of Marquis et al., (2008) the full sequence of all arm choices was analyzed for the number of episodes of spontaneous alternation. An example of the scoring method is shown in Figure 2. The start arm, the left goal arm, and the right goal arms are identified in the example as arms A, B and C, respectively. In this example an animal explored the three arms in the sequence ABCBAC. By analyzing this sequence as 4 overlapping triplet of choices the rat would receive a score of 3 spontaneous alternation episodes. Spontaneous alternation scores were analyzed for healthy controls with independent samples t-test comparing performance between sex (male or female) and HC litter (1 or 2). The LPS rat data was analyzed by examining if their performance fell within the 95% confidence interval of the healthy control data.

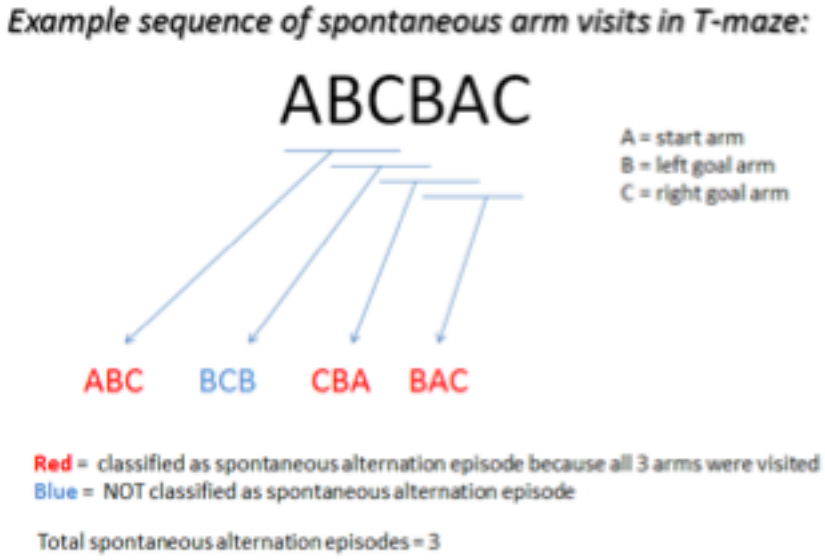

Figure 2. Example sequence of arm visits analyzed in terms of spontaneous alternation episodes

Rewarded alternation training. The healthy control pups that were outliers on the

spontaneous alternation task (visiting considerably more or less arms and having considerably more or less spontaneous alternation episodes) were excluded from the rest of the study to then include 8 pups with an equal number of males and females from each healthy litter. These Healthy Control (HC) offspring from two litters and the 3 surviving LPS-treated offspring served as subjects in the rewarded alternation procedure and all subsequent procedures. Thus, the saline maternal exposed litters consisted of a total of 16 rats, 8 males and 8 females, and the LPS maternal exposed group consisted of 3 offspring, 1 male and 2 females. The rewarded alternation training began on the day after the spontaneous alternation test (Deacon & Rawlins, 2006). The rewarded alternation task consisted of four phases.

Phase 1 Adaptation (1 day). Both goal arms were baited with a 45 mg Noyes sucrose pellet and the rat was allowed to explore the T-maze until it consumed both pellets or 5 minutes had elapsed.

Phase 2 Forced Choice Training (2 days). This phase included 10 trials daily and served to train the rats to go down a goal arm to obtain a food reward. Only one of the guillotine doors was opened leading to the baited goal arm. The arm that was baited on a trial was alternated in a pseudo random fashion with the requirement that the same arm could not be baited on more than 3 consecutive trials.

Phase 3 Alternation Training (6 days). In this phase the rat was trained to alternate between the goal arms on a trial. There were 10 trials per day. The rat ran twice on each trial. On the first run the rat was given a forced choice as in Phase 2 with one goal arm baited. On the next run, which followed immediately, both guillotines were raised and the rat was rewarded only if it chose the opposite arm from the first run.

Phase 4 Delayed Alternation Testing (5 days). The same procedure was used as in the

Alternation Training Phase except that the time between the forced choice on the first run and the choice on the second run was 30 seconds except on the second to last day, when the delay was increased to 60 seconds. The 60 seconds was added to better investigate performance on the alternation task as a function of the delay between the first and second run of a trial. Marquis et al (2008) demonstrated poorer performance on this task when the delay was 30 seconds compared to smaller or no delays. Poorer performance with increasing delays in an alternation task would support the argument that rats were relying on their short-term/working memory to successfully complete the task.

Phase 5 Nicotine treatment (6 days). Nicotine treatment was introduced to obtain additional evidence that rats were relying on their STM to complete the task since nicotine is known to improve performance in STM tasks. Nicotine is usually injected around 15 minutes (Kenny and Markou, 2006; Cohen, Perrault, Griebel, and Soubrie, 2007), and the peak effect is thought to last through 30 minutes (Ghosheh, Dwoskin, Miller, and Crooks, 2001). On the first day, half the rats from both groups received 0.5 mg/kg nicotine and half received saline. Testing took place 12 minutes after injection. On the next day, drug treatment was reversed for all rats. Group averages for nicotine treatment and saline treatment were computed across days. The rats were tested with the nicotine treatment for both rewarded alternation and delayed rewarded alternation.

Dependent variables and statistical analyses. Learning during the various phases of T-maze training was assessed by measuring the *latency* to reach the goal arm and the *percent correct choices*. Initial learning is seen as decreasing latencies to reach the goal arms to obtain the sucrose pellet reward (that is, the rats learn to run faster in anticipation of the available reward). Learning to consistently choose the correct arm during alternation training is seen as a

gradual increase in Percent Correct Choices above 50 % which reflects chance performance.

In all of the data analyses litter and sex was included as an independent variable since the literature suggests that there are sex differences and litter differences in many learning tasks (Varlinskaya, Spear, & Spear, 1999). For example , Health Control (HC) Litter x Sex x Days mixed factorial designs were used to analyze the rewarded alternation training latency data and percent correct choices with healthy control (HC) litter and sex as between-subjects factors and days as the within-subjects repeated measure. This analysis was further analyzed with post hoc tests when significant interactions were obtained. Depending on the phase of training additional independent variables were included in the analysis such as the delay between forced and choice trials or nicotine treatment. A one-sample t-test was used to compare the rats' average performance to chance performance at 50% for percent correct choices in alternation training.

Results

LPS-treatment

There was great difficulty in this study to obtain prenatally LPS-treated rats. Most LPS injections resulted in pregnancy loss. The study started with a dose of 0.5 mg/kg LPS because of previous research (Urakubo et al., 2001) at this dose with successful results. After two pregnancy losses at the original 0.5 mg/kg dose of LPS, both of the previously injected females were given a smaller dose of LPS, one at 0.25 mg/kg, and the other at 0.125 mg/kg of LPS at approximately 15 gestational days of pregnancy. The 0.25 mg/kg induced another pregnancy loss, but there was one lone survivor of the 0.125 mg/kg of LPS.

In order to achieve a greater population of LPS-induced offspring, both of the previously saline-induced females received LPS, one at 0.125 mg/kg, and the other at 0.0625 mg/kg, both

diluted in 1 mg of saline solution at approximately 15 gestational days of pregnancy. The 0.125 mg/kg induced another pregnancy loss, but two LPS-induced offspring were born at 0.0625 mg/kg. The LPS doses needed to be decreased due to pregnancy losses; because of the very small number of surviving rats it is not feasible to look for dose-dependent effects on behavior. The correct dose of LPS-treatment in rats would need to be found in order to create a good model of schizophrenic-like symptoms. Table 1 summarizes which females received either the saline or the LPS and the doses and how many pups were born and survived at each treatment. As a result of the difficulty, the healthy saline-treated rats data will be presented first with confidence intervals as the measure of variability for the spontaneous alternation tasks and standard error bars for the rewarded alternation tasks. The performance of the three surviving LPS-treated rats will then be compared to the healthy saline-treated rats.

Table 1
Pups Born and Survival

	LPS Dose (mg/kg), ip				
Female	Saline	0.5	0.25	0.125	0.0625
H01		0/-		2/1	
H02	11/11				2/2
H03		0/-	0/-		
H04	13/12			0/-	0/-

Note: Pups Born/Surviving Pups. The doses were decreased progressively with at least 3 weeks between doses.

Spontaneous Alternation

The results of the spontaneous alternation task were analyzed with independent samples t-tests to compare performance between the two sexes and HC litters. Figure 3 shows total arms visited (A) and percent alternations (B) in all females and males of both healthy control litters. All graphs plot the means with 95% confidence intervals. The data was trending towards significance between females (M=34.43, SD=8.70) and males (M=24.69, SD=13.31); $t(21)$ =

1.77, $p > .05$, with females visiting more arms than males. There was a significant difference between percent alternations between females (M=61.33, SD=16.85) and males (M=45.39, SD=12.71); $t(21) = 2.51$, p=.02, with females making more spontaneous alternations. Spontaneous alternation behavior is considered to be an indicator of the use of STM. Therefore, these data suggest that the rats were using STM to explore the novel T-maze and females may rely on their STM more than males.

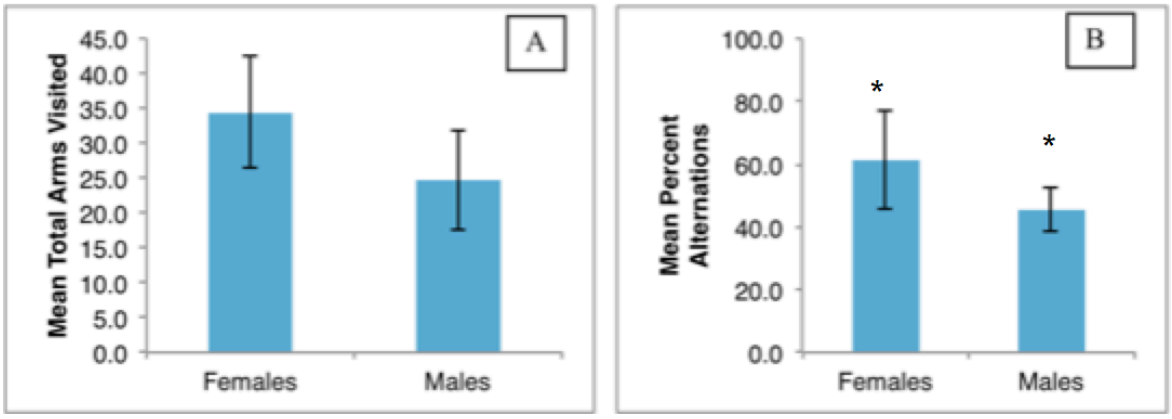

Figure 3. Mean total arms visited (A) and mean percent alternations (B) in male (n = 15) and females (n = 8) from both litters combined. Error bars are 95% confidence intervals.

Figure 4 shows mean total arms visited (A) and mean percent alternations (B) in the two healthy control litters separately. There was no significant difference between HC litter 1 (M=31.08, SD=10.34) and HC litter 2 (M=23.91, SD=14.49); $t(21) = 1.38$, $p > .05$, in total arms visited. There was a significant difference in percent alternations between HC litter 1 (M=56.55, SD=16.34) and HC litter 2 (M=43.36, SD=11.94); $t(21) = 2.20$, p=.04. This means Litter 1 had more spontaneous alternations than Litter 2, showing potential genetic differences in the two healthy control litters.

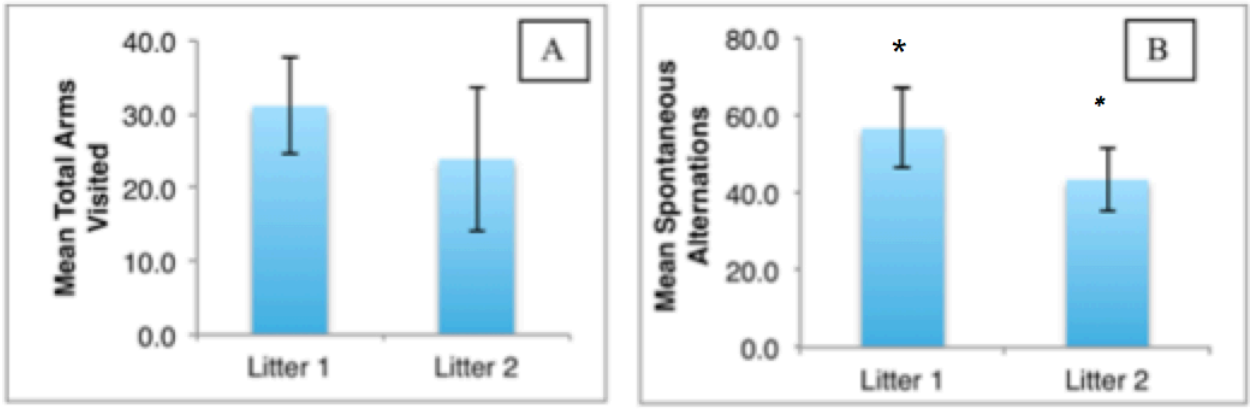

Figure 4. Mean total arms visited (A) and mean percent alternations in HC Litter 1 (n = 12) and HC Litter 2 (n = 11) with both sexes combined. Error bars are 95% confidence intervals.

Alternation Training

First trial latency. One indicator of learning in discrete trial procedures like the T-maze would be a decreasing latency to the goal arms over training days. There were six days of alternation training in total. The first forced trial is where only one arm is available with a food reward. A 2 (HC Litter) x 2 (Sex) x 6 (Days) mixed factorial design was used to analyze the latency data with HC litter and sex as between-subjects factors and days as the within-subjects repeated measure. A significant main effect of days confirms that there was a gradual reduction of latencies of all healthy control pups combined across the six days of training and shows evidence of learning, $F\ (5, 60) = 3.87$, $p = .004$, $n^2 = .244$. However, there was a significant interaction of days and HC litter, $F\ (5,60) = 3.34$, $p = .01$, $\eta_\rho^2 = .218$. Figure 5 shows mean latency across days for litters 1 and 2. This means that the two HC litters differed at some point in the 6 days on their latencies. Post hoc comparisons using the Bonferonni correction test were done to see at which day(s) the two litters differed across days. This interaction was primarily a result of litter 2 completing the task faster than litter 1, on day 5, $t(14) = 2.774$, $p = .015$. A

similar difference between litters on day 4 just missed statistical significance, $t(14) = 2.997$, $p = .066$. There was not a significant interaction between days and sex $F(5,60) = 2.06$, $p = .08$, $\eta_\rho^2 = .147$. This means that over the 6 days, both males and females moved at similar latencies on the first forced trial.

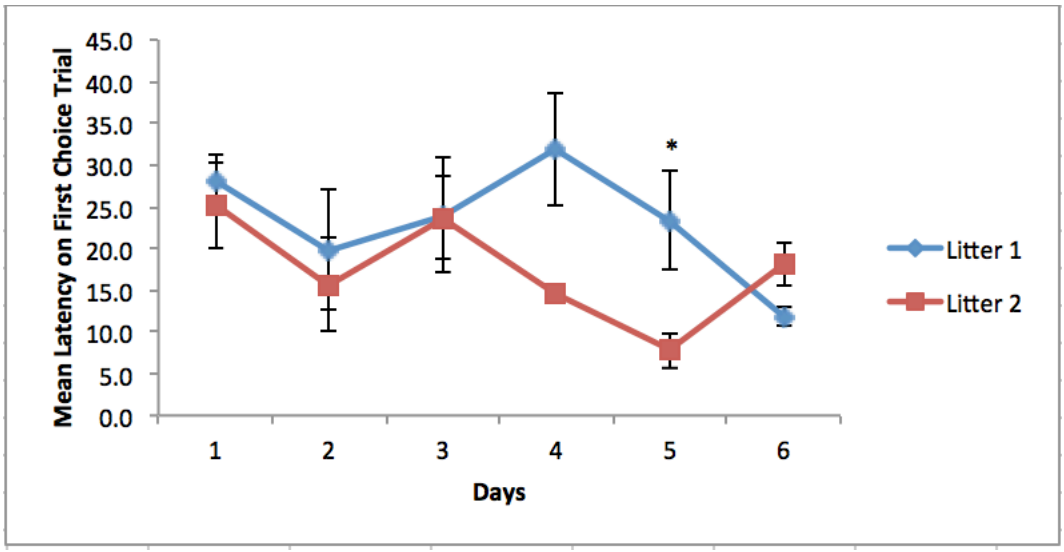

Figure 5. Significant interaction of mean latency on first forced trial across days for litter 1 (n=8) and litter 2 (n=8). Standard error bars are included.

Second trial latency. The second trial is where animals were required to choose the arm opposite of the first forced choice arm in order to receive a food reinforcement on the second trial. Initial learning would be indicated here too by decreasing latencies to the goal arms over days. A 2 (Litter) x 2 (Sex) x 6 (Days) mixed factorial design was used to analyze the latency data with litter and sex as between-subjects factors and days as the within-subjects repeated measure. A significant main effect of days again confirms a gradual reduction of latencies of all healthy control pups combined across the six days of training and shows evidence of learning, $F(5, 60) = 3.62$, $p = .006$, $n^2 = .231$. Figure 6 suggests that Litter 2 showed faster improvement over days than Litter 1. This impression was confirmed by a significant interaction of days and litter, $F(5,60) = 2.72$, $p = .028$, $\eta_\rho^2 = .185$. Post hoc comparisons using the

Bonferonni correction test yielded a statistically significant difference only on day 5, $t(14) = 2.21$, $p = .044$. This was a result of litter 2 completing the task faster than litter 1. There was not a significant interaction of days and sex $F(5,60) = 1.11$, $p = .36$, $\eta_\rho^2 = .085$. This means over the 6 days, both males and females showed similar decreases in latencies on the second choice trial over days.

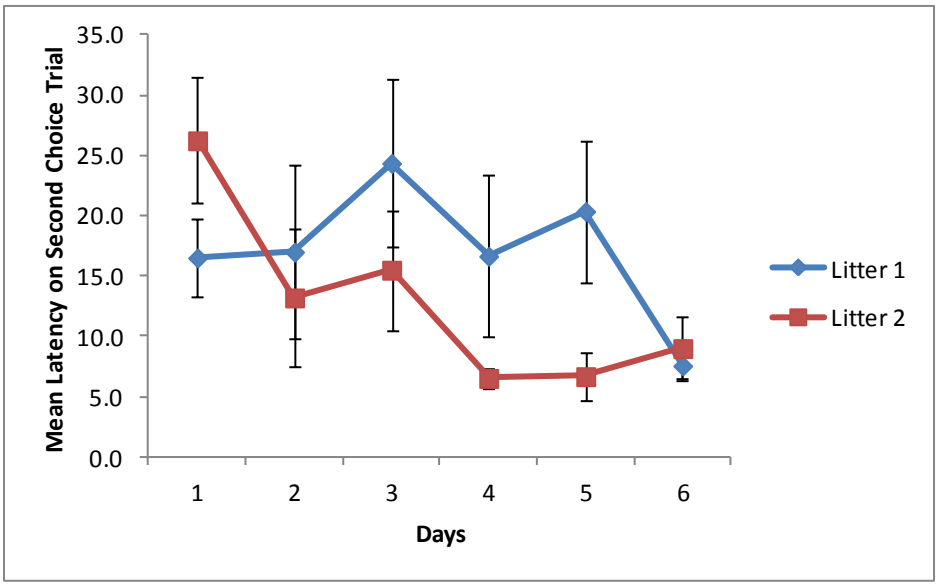

Figure 6. Significant interaction of mean latency on second choice trial across days for litter 1 (n=8) and litter 2 (n = 8). Standard error bars are included.

Percent correct choices. Percent correct choices was measuring whether or not the rats chose the correct arm on the second trial which was defined as the arm opposite of the first forced trial arm. If the animals were learning to rely on their short-term memory to complete the task, the percentage of correct choices should have increased over days. The three-way ANOVA indicated that there was no statistically significant effect of days $F(5,60) = .797$, $p = .56$, $\eta^2 = .062$, and no differences between litters $F(5,60) = 708$, $p = .62$, $\eta_\rho^2 = .056$ and sex $F(5,60) = 1.09$, $p = .38$, $\eta_\rho^2 = .083$. No interactions were significant.

Figure 7 shows that percent correct choices did not decrease over days. Therefore, there is no evidence that the rats were learning to rely more on their short-term memory to perform the task over these training days. However, the animals did seem to be using their short-term memory when compared to chance performance, which is 50%. If no short-term memory was used, the rats should have been choosing the arm at chance levels. The mean performance of all rats combined though was 63%. A one-sample t-test was done comparing the rats' average performance to chance performance. There was a statistically significant effect, $t(16) = 4.19$, $p = .001$. This means that the rats naturally relied on their short-term memory, which was consistent with the spontaneous alternation data. The rats however did not improve in using their short-term memory for this task after 6 days.

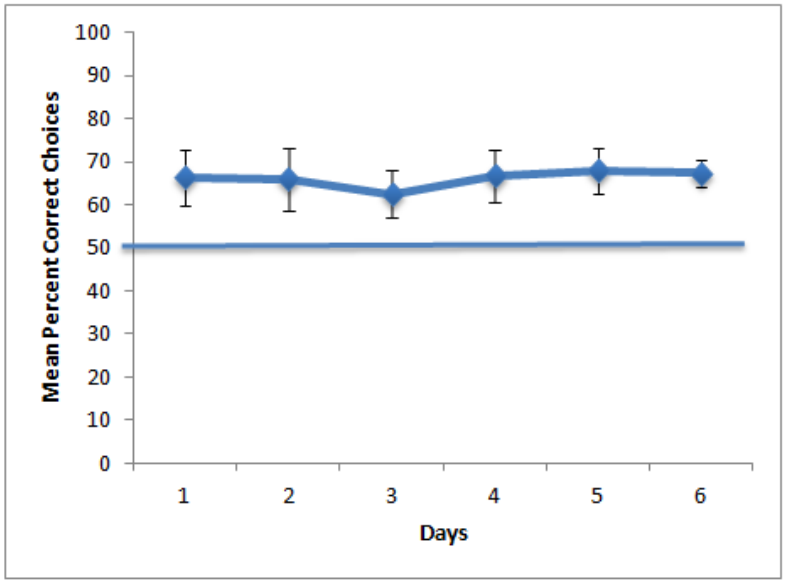

Figure 7. Mean percent correct choices over training days for all rats. Standard error bars are shown. The horizontal solid line at 50% indicates chance performance.

Delayed Alternation Training

Delayed alternation training from days 9-13 were done to see how delays from 30 to 60 seconds would affect performance in the T-maze. If the animals were using their short-term memory to perform the task, their percent correct choice performance should be affected by creating a delay between the first and second trial. The rats were tested for 3 consecutive days at a 30 second delay. To evaluate performance, the average of these 3 days was calculated for each rat. The data from one female rat from litter 2 was not included in this analysis because it did not respond on 2 of these training days. Table 3 shows that at a 30 second delay performance did not decrease towards chance in either litter or sex. The rats seemed to improve compared to the last phase, with an increase in percent correct choices from 63% in the last phase to about 72% in this phase.

On the following day 12, the rats were tested with an increased delay to 60 seconds. Performance was compared to the mean of the 30 sec delay tests with a 2 (Delay: 30, 60) x 2 (HC Litter) x 2 (Sex) mixed ANOVA. The same female was omitted again due to failure of performance on most days. There was a significant main effect of delay, showing performance declined, $F(1,11) = 6.15$, $p = .03$, $\eta_\rho^2 = .358$. When the delay was increased from 30 seconds to 60 seconds, performance did decrease towards chance when looking at the HC rats overall as well as the litters and sex individually (see Table 3). This suggests that the animals were using their short-term memory to complete the task. The variability between the litters in the 60-second delay suggests that there were individual differences in STM ability. Figure 8 shows mean correct choices at the 30 and 60 sec delays for litters and sexes separately; no main effects or interactions between litter and sex were significant.

Table 2

Delayed Alternation Training of HC Rats

	% Correct Choices	
Subjects	30 s Delay	60 s Delay
HC Litter 1	M=71%, SE= 3.6	M=50%, SE= 8.7
HC Litter 2	M=72.7%, SE= 3.7	M=55.7%, SE= 7.5
HC Litters Mean	M=71.8 %, SE= 2.5	M=52.7%, SE= 5.7
Females	M=70%, SE= 4.2	M=50%, SE= 9.8
Males	M=73.3%, SE= 4.2	M=55%, SE= 6.8

Note. HC = Healthy Controls; M = Mean; SE = Standard Error; s = seconds

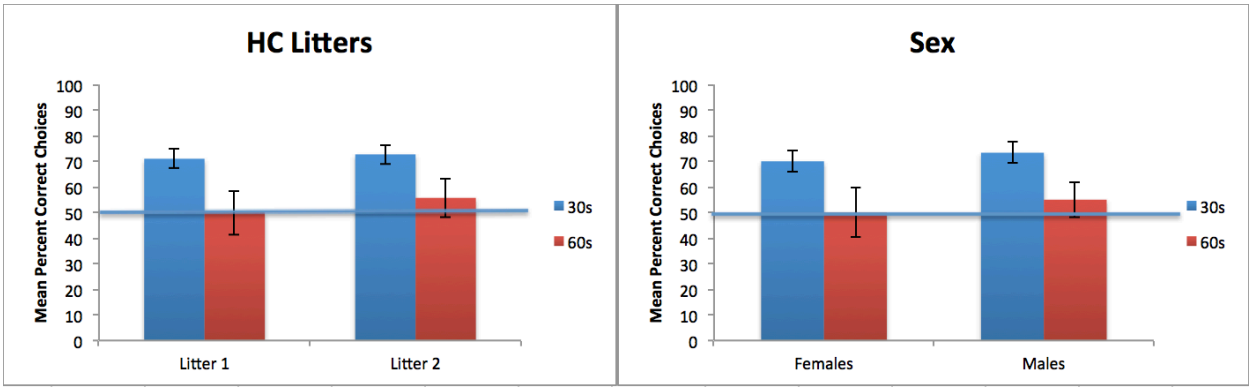

Figure 8. Mean percent correct choices of litters at 30 and 60-second delays, and mean percent correct choices in males and females at 30 and 60 second delays. Standard error bars are shown.

Nicotine Treatment

It has been shown that nicotine improves short-term memory performance (Carlson et al., 2009). The final part of this experiment was to determine if nicotine could improve performance and if there was a difference between performance across litters and sex. The nicotine treatment data were analyzed using a 2 (Drug: Nicotine, Saline) x 3 (Delay: 0, 30, 60) x 2 (HC Litter) x 2 (Sex) mixed design ANOVA with Drug and Delay as within-subjects factors and HC Litter and Sex as between-subjects factors. Figure 9 shows a main effect of delay $F(2,20) = 6.61$, $p = .006$,

$\eta_p^2 = .398$. This indicated that increasing the delay affected overall performance. Additional pairwise comparisons indicated that performance at the 60-second delay was worse than at 0-second delays, but performance at the 30-second delay did not differ from the 0-second delay. The rats' performance no longer dropped to chance at the 60-second delay, showing that the rats improved during this nicotine treatment phase as a result of continued testing in the task. Nevertheless, these results provide evidence that short-term memory was being utilized because the delay had an effect.

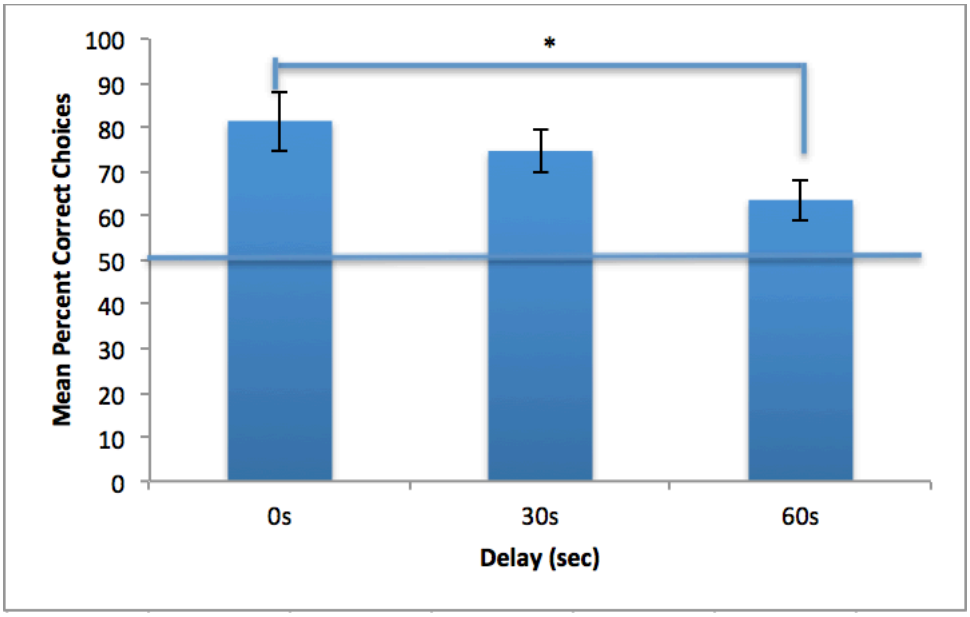

Figure 9. Main effect of delay of mean percent correct choice. Standard error bars are shown. The horizontal line at 50% shows chance performance.

However, Figure 10 shows that nicotine treatment did not affect performance in the task. The main effect of drug was not statistically significant, $F(1,10) = .168$, $p > .05$, $\eta_p^2 = .017$ and there were no significant interactions with the drug as a factor. This means the expectation that nicotine will improve performance was not supported.

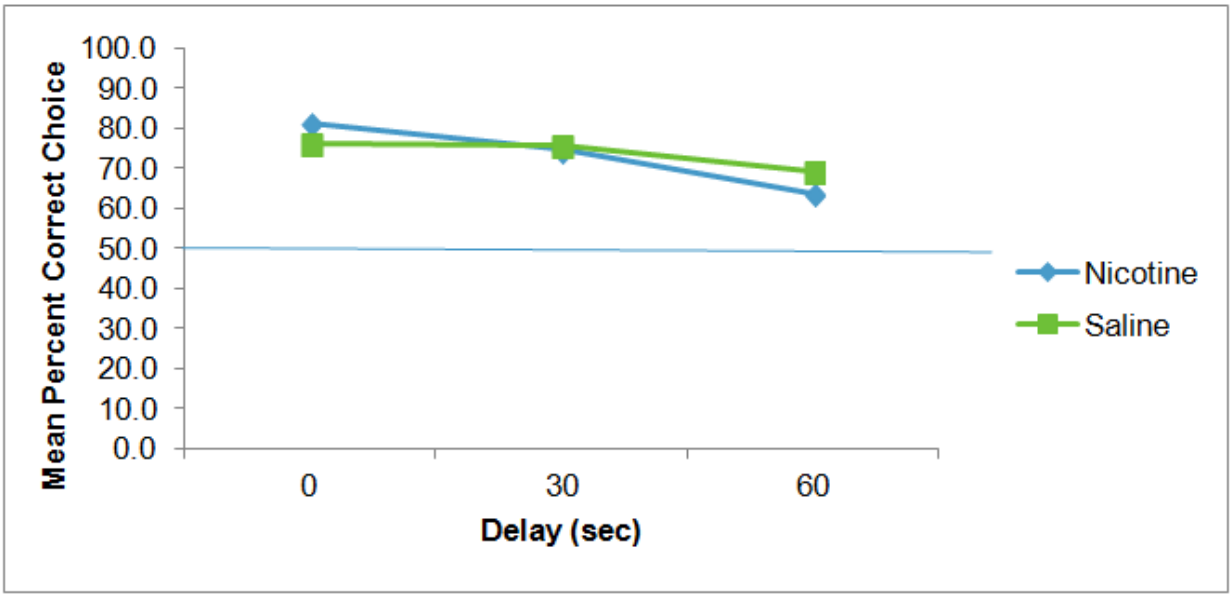

Figure 10. No effect of drug on mean percent correct choice across delays. The horizontal line at 50% shows performance at chance.

Figure 11 shows that there was no main effect of litter $F(1,10)=3.05$, $p>.05$, $\eta_\rho^2=.995$, but there was a statistically significant main effect of sex $F(1, 10)= 13.51$, $p=.004$, $\eta_\rho^2=.575$. Higher percent correct choices were made by females compared to males throughout the nicotine treatment phase. This shows that the females seem to be using their short-term memory more than the males and is consistent with the results from the previous phases of this experiment.

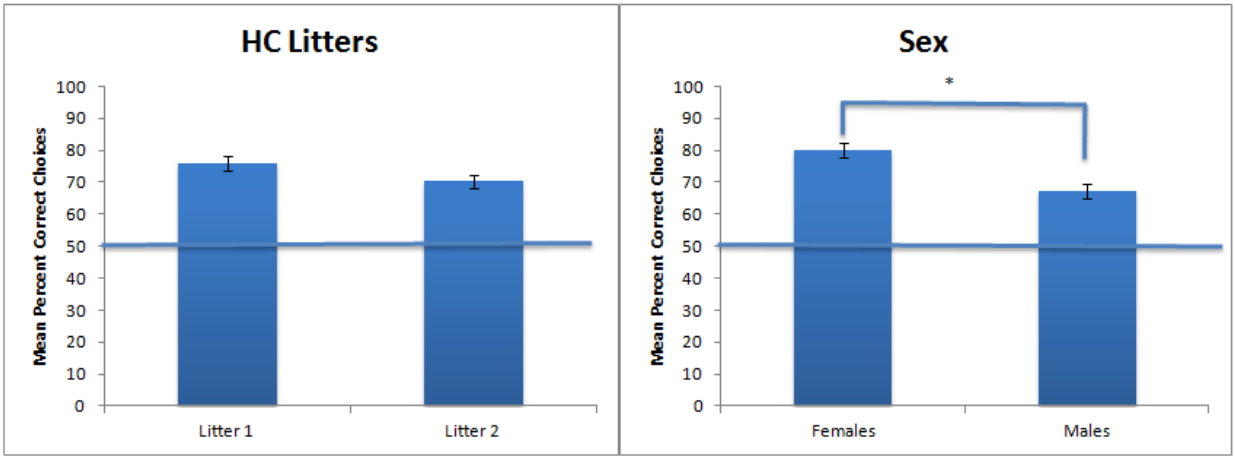

Figure 11. No effect of litter, main effect of sex on mean percent correct choices. Standard error bars are shown.

Offspring of LPS-Treated Rats

The mothers of these rats were treated with LPS during pregnancy and the pups were later tested on the same tasks mentioned above for the healthy control rats. There were three LPS-treated rats from two different litters (see Table 1).

Spontaneous alternation. The spontaneous alternation results are summarized in Table 3. The three LPS-treated rats on average visited 28.7 arms. This is compared to the healthy rats at an average of 27.7 (95% CI [22.1, 33.2]). The LPS-treated rats had a mean of 50.2 percent correct alternations, which was the same as the healthy rats. This shows that the number of arms visited and percent spontaneous alternation for the LPS-treated rats fell will within the normal range of responding of the healthy saline-treated rats.

Table 3

Spontaneous Alternation Testing of LPS-treated rats Compared to Healthy Saline-treated Rats

Subjects	Arms Visited			% Spontaneous Alternations		
LPS1-m	15			47.0		
LPS2-f	44			59.5		
LPS3-f	27			44.0		
ALL LPS rats	28.7			50.2		
		Confidence Interval			Confidence Interval	
		LB	UB		LB	UB
All Healthy Rats	27.7	22.1	33.2	50.2	43.5	57.0

Note: LB = Lower Bound ; UB = Upper Bound

Alternation Training. The male LPS-treated rat had a mean time of 50.4 seconds on the first forced trial, and the two females from the second litter had a mean time of 53.59 seconds. The first rat had a mean time of 46.4 seconds on the second trial, and the two females had a mean time of 52.04 seconds where a choice was made. This long latency (recall that the time limit for obtaining a reward during a trial was 60 sec) shows that these rats did not learn to run quicker to the sucrose reward during training. The male rat made at least one choice on 5 out of the 6 days. This means that he did not enter the goal arm in the first forced trial at all on one of the days. Out of the 5 days that the male rat made it to part two of the trial, he only actually completed a choiceon 3 of those 5 days. On the other 2 days the male rat sat in the start arm for 60 seconds on each of the times he made it to part two of the trial, therefore never making a choice. On those 3 days that he made it to part 2 of the trial and actually made a choice, he received 100 percent correct choices. The first female only made choices on 1 day getting 100 percent correct choices. The second female made choices on 3 days, getting 0 percent correct choices on two days and 100 percent correct choices on the third day. This is not an accurate measure to be

compared to the healthy controls at 63% though, because the LPS-treated rat would only complete a few trials out of the 10 as described above. These results suggest that maternal treatment with LPS profoundly disrupts performance on forced alternation training, since no healthy rat showed this lack of performance.

Delayed Alternation Training. The first male LPS-treated rat had a percent correct alternation of 80% at the 30s delays not completing all 10 trials, and only completed one choice of the 10 trials on the 60s delay day. The females from the second LPS-treatedlitter only completed the first 3 days at the 30s delay due to time constraints, but interestingly performed a little more. The second litter had an average of 63.5% percent correct choices at the 30s delay. However, these data still cannot be accurately compared to that of the HC because the percentages were not accurate measures of short-term memory for the LPS-treated rats. This is because many of the trials were not completed and the LPS-treated rats needed to have accurately learned the alternation training task to make these results more accurate.

Nicotine treatment. Despite the poor performance during delayed alternation training the LPS-treated male rat was tested with nicotine to see if nicotine treatment (and additional training) would improve performance in the T-maze task. However, performance continued to be inconsistent making it difficult to make any conclusions concerning the use of short-term memory during the task. The female LPS-treated rats were not tested with nicotine.

Discussion

The hypothesis that the LPS-treated rats will differ in performance of spontaneous alternation, alternation training, delayed alternation training, and nicotine treatment, due to poorer short term memory, could not be adequately tested in this present study because of the

low number of LPS animals as result of a high rate of pregnancy loss. However, the spontaneous alternation of the LPS-treated animals was normal. This indicates that their short-term memory was still sufficient in helping them with their natural inborn strategy to search new areas, which was still intact. Despite good performance in the spontaneous alternation training, performance in the rewarded alternation tasks was severely disrupted. Hunger motivation should not have been an issue because all the healthy control and LPS-treated rats were food deprived. It was observed anecdotally that the 3 LPS-treated rats never climbed on the cages nor tried to jump out of a cage. All of the healthy control rats were very active in this aspect. The LPS-treated rats also consistently took the full time for their alternation training, and did not always reach the ends of the arms in the T-maze to eat the pellets. They were moving around, not just sitting in the start arm, but when they started to move into a goal arm they were very hesitant. Other kinds of learning are needed for performance in the rewarded alternation tasks that are independent of the need to utilize short-term memory, thus these animals may have been displaying other learning deficits despite normal STM. Nevertheless, despite food deprivation, a lack of sufficient motivation to obtain the sucrose reward pellets cannot be ruled out as an explanation for the poor performance of the surviving offspring of LPS-treated pregnant rats.

Since the LPS-treated animals never learned the instrumental task, they could not be tested for short-term memory deficits in the delayed alternation training and the nicotine phases of this experiment. It was hypothesized that cognitive deficits would be seen in the LPS-treated rats due to neuroinflammation of the brain during development (the cytokine hypothesis) and therefore modeling the cognitive symptoms of Schizophrenia (Ratnayake et al., 2013). Many other behavioral deficits were observed though suggesting that it may be worthwhile to pursue additional research to determine if LPS-treated rats are a good model to measure Schizophrenic-

like cognitive symptoms. Due to these complications the main hypothesis could not be tested adequately. The results of the experiment however can address the usefulness of the T-maze as a measure of short-term memory.

The spontaneous alternation task showed that females made significantly more alternations than the males in the healthy control group. Since both groups did spontaneously alternate, it can be assumed that short-term memory was being utilized, but that females may use their short-term memory more so than males. There have also been sex differences due to maternal prenatal stress in a water-maze (Nishio, Kasuga, Ushijima, and Harada, 2001). The present study therefore confirms prior observations that sex differences exist in performance during a learning task. All the healthy control pups showed spontaneous alternation in the T-maze. However, there were significant differences observed between litters. Litter 2 of the healthy control pups made significantly more percent alternations than litter 1 of the healthy control pups. These differences may be due to inherited characteristics that contribute to individual differences in performance on a task. It has been suggested that developmental research designs with rats should not include groups with more than one animal from the same litter (Varlinskaya, Spear, & Spear, 1999). The present study shows that this is an important point and should be considered in all developmental designs with rodents.

The alternation training showed that all healthy control rats ran the T-maze increasingly faster across the 6 days in both the first forced trial and the second choice trial. (This learned performance over days was never observed in the LPS-treated rats.) The healthy rats did not show improvement in the percent correct choices during the first 6 days of training, however their performance across the 6 days was significantly greater than chance, which is 50%. This result confirms previous research that rats show a natural tendency to alternate during

spontaneous and rewarded alternation tasks (Gaffan & Davies, 1982). Since the healthy control rats performed above chance in the alternation training, indicating use of their short-term memory, the present study included a delayed alternation task. This task included a 30-second delay, as well as a 60-second delay. The purpose of these delays were to see if a limit could be imposed on short-term memory causing performance to be reduced to chance at 50%. For example, in the no delay condition it is possible that the rats were using another strategy like following their most recent trail smell or some other unknown strategy. Increasing the delay is predicted by a short-term memory hypothesis to reduce performance. The results showed that at a 60-second delay between the first forced choice and the second choice training the performance dropped down to about chance. Therefore, we know for certain that the rats were using short-term memory because the delay placed greater demand on the rats and resulted in a drop in performance.

The rats relied on their short-term memory to make the correct goal arm choice from the very first day of alternation training, but any improvement in their utilization of STM (i.e., increases in percent correct choice performance) was not observed during the first 6 days of alternation training. They did improve though when looking at their performance across all training phases. By the nicotine phase the rats were performing well above their initial performance and well above chance even at the 60-second delay.

It is well known that the majority of people with Schizophrenia smoke, possibly due to self medication (Kumari &Postma, 2005). Nicotine is known to improve short-term memory (Sacco et al., 2005). This phase of the experiment was to see if nicotine could significantly decrease the cognitive deficits from LPS-treatment. Although this aspect of the study could not be completed because of the failure of LPS-treated rats to learn the task, improved performance

in nicotine-treated healthy control rats would provide additional support that STM was being utilized by the rats and that STM could be experimentally manipulated in the T-Maze task. However, there was no significant difference between the nicotine and saline treatments in the healthy control rats. Unfortunately, we were able to test only one dose of nicotine in the present study. A proper test of any nicotine effect on performance would require tests at many doses to obtain a dose-response curve.

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