BEHAVIORAL EFFECTS OF CHRONIC RISPERIDONE TREATMENT ON JUVENILE MALE RATS

by

Lindsey A. Boman

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree

MASTER OF ARTS

Major Subject: Psychology

West Texas A&M University

Canyon, Texas

December 2017

ABSTRACT

Despite substantial increases in the use of antipsychotics, there is a lack of literature regarding the long-term effects of early treatment. Some studies have indicated that early administration results in differential alterations to neurotransmission systems, but few studies have been conducted to investigate the long-term behavioral modifications. Therefore, the aim of the current work was to examine the behavioral effects of low dose risperidone (a commonly used antipsychotic) treatment in rodents. Twenty-four male Sprague-Dawley rats were randomly assigned to either a subcutaneously implanted continuous release risperidone treatment (.04 mg/day) or a sham pellet condition. To encompass the peri-adolescent to adolescent time frame (postnatal days 40-60), thought to be important for brain development (Schneider, 2013; Spear, 2000) male rats began risperidone treatment at post-natal day 35. Following a 6-week treatment period, adult rats (Wiley, 2008), were given a battery of behavioral assessments. No significant differences were found between groups in the Open field, Object recognition, Spatial recognition or Morris water maze tasks. Additionally, Y-maze yielded no differences in percentage of spontaneous alternation and alternate arm return patterns. However, significant differences were found between groups in the number of same arm returns,

which has been proposed to be indicative of working memory deficits. Since this is likely the first study of its kind using this route of administration, more work needs to be done to determine if early exposure to risperidone may lead to differences in spatial working memory in adulthood. However, these findings seem to indicate that early low dose risperidone treatment does not severely impair behavior in later adulthood.

ACKNOWLEDGEMENTS

Firstly, I want to thank my God who has never left my side, and without whom, I would not have had the strength, knowledge or courage to complete this journey.

To my committee chair person, Dr. Maxine De Butte-Reardon, who has not only made this project possible, but has graciously guided me through it and the entirety of my educational career, with unfailing patience and kindness. I cannot thank you enough for believing in me and for everything you have done to help me throughout this project.

I would also like to express my sincere gratitude for my committee members, Dr. Denton, Dr. Byrd, and Dr. Green for all for the time, feedback, support, and encouragement you have given as I worked my way through this study.

I would like to thank my mom Rebecca Weatherford for always being there for me no matter the circumstances, and for continuously showing me through her own accomplishments that with hard work and persistence, I can achieve any goal.

To my family and friends, I cannot thank you enough for all the love, laughs, advice, and support you have provided me with throughout my academic career.

To the best technology professor I know, my aunt Karen Boatman, thank you for your unyielding pursuit to keep me and my computer going throughout my thesis and educational career.

To my best friend and colleague, Blake Gieseking I am so thankful for every high and low we have confronted together. I could not imagine what it would have been like to face this challenge without the help, humor, feedback and support you have provided me with during this thesis and throughout our education.

I would also like to acknowledge Jason Rodin for lending his expertise and time. His tireless pursuit to educate me on how to deal with statistical violations has truly given me deeper understanding of the statistical findings, research methods and what it means to be a responsible statistician. I am so grateful for your help and friendship.

I would also like to thank Dr. Atchison, Dr. Fisher, Dr. Byrd and Dr. Denton for instilling in me the importance of ethical practice, the necessity of always weighing the cost against the potential benefit of treatment, and for inspiring me to challenge my pre-conceived assumptions about current practices. Without this background, I may not have had an interest in the current research. I have no doubt that I will be a more ethical clinician, thanks to the wisdom you have imparted in me.

Lastly, I am dedicating this thesis to my loving husband, Brad Boman and children, Brooklyn and Brayden Boman whom have inspired me to become the best part of myself and have given me unceasing support, strength and understanding as I worked countless hours to obtain this goal.

Approved:

Chairman, Thesis Committee	Date
Member, Thesis Committee	Date
Member, Thesis Committee	Date
Member, Thesis Committee	Date

Head, Major Department	Date
Dean, Academic College	Date
Dean, Graduate School	Date

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CHAPTER I

INTRODUCTION

In the last 15 years, there has been an alarming increase in the use of antipsychotics (AP) for treating children with a variety of psychiatric conditions (Masi et al., 2015). A survey conducted by Olfson, Blanco, Lui, Wang, & Correll (2012) reported a 750% increase in AP use among children inside the United States from 1993 to 2009. While studies have indicated that treatment may help alleviate some of the symptoms associated with schizophrenia, pervasive developmental, bipolar and attention deficit disorders (Zuddas, Zanni & Usala, 2011), they have also shown that youths are at a greater risk of harmful side-effects associated with AP administration (Menard, Thummler, Auby, & Askenazy, 2014).

This may be problematic because recent studies have found that individuals who begin AP's during childhood tend to remain on these medications for long periods of time (Memarzia, Tracy & Giaroli, 2016). Risperidone (also known as Risperidal) is the most commonly administered AP in the pediatric population (Bardgett et al., 2013; Memarzia, et al., 2016) and its therapeutic efficacy comes from alterations to the neurotransmitter systems in the brain (Bardgett, et al., 2013). Unfortunately, the neurotransmitters that are

implicated in AP use are also important for a multitude of other developmental processes (Milstein et al., 2013; De Santis, Lian, Huang & Deng, 2016). Therefore, early prolonged exposure to AP's may raise the risk of long-term neurodevelopmental changes in a child's still developing brain (Memarzia, Tracy & Giaroli, 2016).

Despite the increased risk and longevity associated with treatment in this population, few studies have been conducted to determine the long-term safety of risperidone (Masi et al., 2015). The gap encountered in the literature is likely due to the ethical limitations and increased environmental variability involved in pharmacological studies with this age group (Bardgett, et al., 2013; Menard, et al., 2014). Therefore, clinical studies using rodent models are heavily relied on to reduce inconsistencies between subjects and to better understand the effects AP can have on a developing population (Andersen & Navalta, 2011; Bardgett et al., 2013).

Prior rodent studies have indicated that long-term alterations to neurotransmitter receptors may occur after early exposure to psychotropic medications (Andersen, 2003; Andersen & Navalta, 2004; Maciag et al., 2005). Cognition and behavior could possibly be affected as a result of these developmental changes (Mandell, Unis & Sackett, 2011; Milstein et al, 2013; De Santis, et al., 2016). However, the majority of research in this area has been conducted with older rat populations and has been riddled by limitations due to small sample sizes, insufficient methodologies, and genetically altered species (Bachmann, Lempp, Glaeske, & Hoffmann, 2014; Hutchings, Waller, & Terry, 2013; De Santis et al., 2016).

Therefore, little is known about whether early AP use results in long-term neurobehavioral consequences (Daviss, Barnett, Neubacher, & Drake, 2016; Zuddas, et al., 2011). The probable effects associated with the use of AP's in rodents may help us better understand the behavioral risks posed to children after early administration (Andersen & Navalta, 2011). Therefore, these potential effects merit careful investigation. As such, the current study examined whether there were any behavioral changes following chronic risperidone administration in male juvenile rats.

CHAPTER II

LITERATURE REVIEW

The Development of Typical and Atypical Antipsychotic Medications

Antipsychotics (AP) are drugs typically used to alter mood and behavior by manipulating several neurotransmitter systems, especially dopaminergic and serotonergic transmission (Ferreira et al., 2016). They were first developed in the 1950's, with the discovery of chlorpromazine. Chlorpromazine was originally used to tranquilize individuals during psychotic episodes. Later, it was discovered that this drug had beneficial effects and could be used to help patients live more autonomous lifestyles and improve their overall quality of life.

A novel class of drugs known as typical antipsychotics (first generation) was soon introduced. These drugs worked as antagonists, primarily to the D_2 and D_3 receptors and included drugs such as haloperidol. The antagonistic action on the dopaminergic system is likely the reason for its therapeutic efficacy and can be used to help relieve some of the positive symptoms (hallucinations and delusions) often seen in children who have schizophrenia (Bardgett, et al., 2013). However, major changes to the dopaminergic

system can also facilitate harmful side-effects. For instance, a reduction in dopamine from the substantia nigra to the dorsal striatum has been linked to the presence of

Parkinsonian-like symptoms and a decrease of motor control that is sometimes experienced by children after treatment with AP medication (Johnstone, Frith, Crow, Carney, & Price, 1978; Memarzia et al., 2016).

To address this issue, atypical AP (second generation) such as clozapine, risperidone, olanzapine and quetiapine were introduced (Ferreira et al., 2016). These medications typically act on serotonin pathways by blocking serotonin 5-HT^{2A} and by stimulating the 5-HT^{1A} receptors (Kellendonk et al., 2006; Lian, Pan & Deng, 2016). Similar to its predecessor, atypical AP also hinders dopaminergic transmission, however, to a lesser extent (Ferreira et al., 2016; Moran-Gates et al., 2007). For this reason, atypical AP are thought to be more beneficial due to their reduced association with extrapyramidal side effects and increased neurocognitive benefits, particularly in patients with the diagnosis of schizophrenia (Andersson, Hamer, Lawler, Mailman, & Lieberman, 2002; Ferreira et al., 2016).

Although Atypical AP's are grouped within the same class of drugs, their mechanism of action including differential patterns of receptor occupancy are quite different from one another (Andersson et al., 2002; Farrelly et al., 2014). The inconsistency that occurs between the drugs within this class may account for some of the variance occurring between atypical antipsychotics in terms of neurodevelopmental (Lian et al., 2016), behavioral (Andersson et al, 2002; Baker, Florezynski, & Beninger, 2015,

Lian et al., 2016; De Santis et al., 2016) and structural differences in the brain (Vernon, Natesan, Modo, & Kapur, 2011).

For example, Andersson et al. (2002) found that clozapine produced significant increases caudate-putamen volume while animals treated with olanzapine showed significant decreases in that same area following eight months of drug administration (Andersson, et al., 2002). Conversely, animals treated with risperidone showed no significant structural differences to the caudate-putamen compared to the controls. These findings indicate that structural effects of AP use are drug specific (Andersson et al., 2002; Vernon et al., 2011;).

Of all second-generation AP's, risperidone is the medication most commonly prescribed in the pediatric population (Bardgett et al., 2013; Memarzia et al., 2016). It has considerable affinity for serotonin $5HT_{2A}$ and D_2 dopamine receptors (Schotte et al., 1996; Toren, Ratner, Laor, & Weizman, 2004). However, it has an even greater attraction to serotonin receptors with a ratio of 8:1 and also binds with other receptors such as, D_1, D_3 D_4 histamine H_1 and adrenoceptors α_1 and α_2. Risperidone has been approved by the FDA to treat symptoms of bipolar mania in adolescents (Krieger & Stringaris, 2013) and irritability associated with autism (Politte & McDougle, 2014). However, it is often prescribed off label to children displaying other diagnoses (Memarzia, et al., 2016).

The recent widespread prescribing and use of these medications has facilitated a large debate among researchers and clinicians (Hutchings et al., 2013; Huybrechts et al., 2012; Seida et al., 2012). This controversy centers around the scarcity of controlled

clinical trial data on the effects of long-term treatment in young populations. It has been argued that generalizing the behavioral findings of AP treatment of adult rodents onto the juvenile population may not take into account effects these drugs may have on the developing brain (Andersen & Navalta, 2004; Menard et al., 2014).

Neurodevelopmental Effects of Antipsychotic use on Neurotransmitter Systems

Dopamine (DA) and serotonin (5-HT) systems are imperative in the regulation of many neurodevelopmental processes (Frost & Cadet, 2000; Levitt, Harvey, Friedman, Simansky, & Murphy, 1997). The serotonergic system develops at an earlier time in the life cycle, while the DA system appears to have a more prolonged developmental course that is dependent on the particular area of the brain that is implicated and receptor sub-type (D_1, D_2, D_3, D_4) (Lambe, Krimer, & Goldman-Rakic, 2000; Vinish et al., 2013). For instance, dopamine D_1, D_2 and D_4 receptors in the frontal cortex are not fully developed until late adolescence.

As discussed, all second-generation AP's including risperidone work on 5-HT receptors in some way, which in turn interact with the dopaminergic system within the thalamic-striatal-frontal loop (TSFL) (Kellendonk et al., 2006; Lian et al., 2016). The TSFL is believed to be important for cognitive flexibility (Cools, 2006), reward based learning (Schultz, 2002) and memory (Kellendonk et al., 2006). Therefore, artificial changes to these neurotransmitters due to early administration of AP may leave the DA and 5-HT systems at greater risk of neurodevelopmental alterations than their adult

counterparts (Frost & Cadet, 2000; Levitt et al., 1997; Lian et al., 2016; Mandell et al., 2011; Milstein et al., 2013; Tarazi & Baldessarini, 2000).

Moran-Gates et al. (2007) conducted a study to determine if there were any alterations in dopamine (DA) receptors within the forebrain region of juvenile (postnatal day 30) compared to adult rats (postnatal day 90) following long-term risperidone exposure at multiple dosages. Twenty-four male rats were randomly assigned to one of three risperidone treatment groups; 0.3, 1.0 or 3.0 mg/kg per day or a saline control group beginning at postnatal day 22. Coronal sections of the medial prefrontal, nucleus accumbens, cerebral cortex, hippocampus and caudate putamen of the rats' brains were taken on postnatal day 42. These regions were chosen because they have major implications in cognitive, locomotor and emotional functioning. Samples of the brain were observed using *in vitro* receptor autoradiography and image analysis.

The findings of this study were compared to previous research regarding the effects of long-term risperidone use in adult rat brains at the same dosages as seen in the aforementioned study, following four weeks of subcutaneous treatment though an osmotic mini pump (Tarazi, Zhang, & Baldessarini, 2001). Juvenile rats treated with risperidone at 1.0 and 3.0mg/kg daily had a significant increase of D_1 receptors in the nucleus accumbens and caudate-putamen (Moran-Gates et al., 2007). This effect was not evident in the adult rat population. However, in both the adult and juvenile rat groups, long-term administration of risperidone increased D_2 receptors in a dose-dependent manner within the prefrontal cortex and hippocampus. Despite this effect on both groups,

the 3.0 mg/kg showed greater efficacy of enhancing D_2 binding in the juveniles (90%) compared to adults (30%). Lastly, D_4 labeling in the caudate-putamen was also increased within both age groups for all three dosages. These findings suggest long-term treatment with risperidone can have dose-dependent effects on dopamine receptor binding in brains of juvenile rats and that young animals may be more vulnerable to any cerebral effects that may occur due to chronic risperidone use.

In accordance with the aforementioned research, Lian et al., (2016) also looked at the effects of how early exposure to aripiprazole, olanzapine and risperidone affected serotonin and dopamine receptor binding in male and female rats and combined these findings to previous studies looking at similar treatment effects in adult rats. On postnatal day 23, rats were randomly assigned to aripiprazole, olanzapine, risperidone or a vehicle control group. The aripiprazole and olanzapine treatment rats received 1.0 mg/kg of the medication while the risperidone group were given 0.3 mg/kg. The drugs were administrated three times daily in water and cookie dough powder for 20 days. Following a two-day wash-out period after the final drug treatment, the rat's brains were removed, and coronal sections were taken. Using quantitative autoradiography, 5-HT and DA receptor binding densities were examined.

Analyses of the findings indicated that the olanzapine group showed reduced binding density of the 5-HT_{2C}R and the 5-HT_{2A}R within the prefrontal cortex of both male and female rats. Therefore, olanzapine may elicit comparable effects within the serotonergic system in juvenile and adult populations. However, it was noted that early

treatment with olanzapine does lead to differences in glutamate and GABA levels in the nucleus accumbens (Xu, Gullapalli, & Frost, 2015) and dendritic form (Frost, Page, Carroll, & Kolb, 2009). Additionally, an increase in D_2 receptor binding was found in male rats juxtapose to the female rats who displayed a decrease (Lian, et al., 2016). A sex difference was also documented in the cingulate cortex, where female rats showed a significant increase of D_1R within the cingulate cortex compared to the males.

Similarly, female rats in the risperidone treatment group also showed a reduction in 5-$HT_{2C}R$ and 5-$HT_{2A}R$ binding density in the prefrontal cortex of the young rats. However, to date no known comparative study has been done in order to see if female rats in adulthood have similar effects to 5-HT binding after chronic risperidone use. Male rats in the risperidone treatment group presented with reduced D_1R bindings in the prefrontal and cingulate cortex compared to adults. No significant differences were found in D_2R receptor bindings in the striatum. This outcome was consistent with Kusumi et al (2000) who reported that 6-week-old male rats given risperidone for 3 weeks showed no changes to the D_2R binding in the striatum.

Additionally, male rodents showed decreases in 5-$HT_{2C}R$ binding as well as 5-$HT_{2A}R$. These findings converge with previous studies in which young male rats who were given 0.3, 1.0 and 3 mg/kg of risperidone for three weeks showed significant decreases in 5-$HT_{2A}R$ bindings in the PFC and dorsolateral-frontal cerebral cortex of both juvenile male rats (Choi, Moran-Gates, Gardener, & Tarazi, 2010) and adult rats (Tarazi, Zhang, & Baldessarini, 2002). However, since low dose risperidone 0.3mg/kg given to

juveniles has the same cortical effect as the higher dosages 3 mg/kg on the 5-HT_{2A}R in adult male rats, it could be hypothesized that the younger rats may be more sensitive to the effects of risperidone treatment (Lian et al., 2016).

In conclusion, the above-mentioned findings indicate that drugs such as risperidone may alter serotonergic and dopaminergic neurotransmission of young rats in a dose dependent manner (Moran-Gates et al., 2007). Although early use of other atypical AP such as olanzapine may not lead to developmental differences in serotonin receptor bindings, (Lian et al., 2016) risperidone does seem to have a greater effect on the young rat brain and these effects may occur at lower dosages than that of the adult population (Choi, et al., 2010; Lian et al., 2016). Thus, rodents receiving risperidone at a young developmental age, may be at greater risk of enduring neurodevelopmental differences (Lian et al., 2016; Milstein et al., 2013; Moran-Gates et al., 2007; Piontkewitz, Arad, & Weiner, 2011; Qiao et al., 2014; Qiao, Zhang, & Li, 2013). These alterations in neurotransmitter systems are thought to contribute to differing behavioral outcomes (Castellano et al., 2009; Lalonde, 2002; Wahlstrom, White, & Luciana, 2010; Wahlstrom, Collins, White, & Luciana, 2010) and may lead to enduring behavioral alterations (Castellano et al. 2009; Hutchings et al., 2013; Karl, Duffy, O'Brien, Matsumoto, & Dedova, 2006; Qiao et al., 2013; Schneider, 2013; Stevens, Gannon, Griffith, & Bardgett, 2013; Terry et al., 2003; Wiley, 2008; Xu, Gullapalli, & Frost, 2015).

Neurobehavioral Effects of Risperidone in Rats

Due to the ethical limitations involving pharmacological studies with children, rodents are often used to allow researchers to yield information regarding the potential positive and negative effects of psychiatric medication use (Bardgett et al., 2013; Andersen & Navalta, 2011). Despite evidence indicating young rats are more susceptible to neurodevelopmental effects of risperidone (Choi, Gardner & Tarazi, 2009; Moran-Gates et al., 2007; Lian et al., 2016; Milstein et al., 2013; Qiao, et al., 2013; Piontkewitz et al., 2011; Schneider, 2013) few pre-clinical trials have been done to assess potential behavioral effects of chronic administration of risperidone using juvenile rodents (Bardgett et al. 2013; Castellano et al., 2009; Andersen & Navalta, 2011; Stevens et al., 2016). Although the research is limited, some studies have been conducted to assess how risperidone may affect young rats' locomotor, exploratory, anxiety-like, spatial learning and memory behaviors.

Locomotor, exploratory and anxiolytic-like effects. Castellano et al. (2009) conducted a study looking at the behavioral and morphological effects of chronic (140 day) risperidone, on male Wistar rats. Twenty-four rats who were four to five weeks old, were randomly divided into one of two treatment groups. Twelve rats were assigned to a risperidone treatment group in which they received 1mg/kg of medication per day in their drinking water. The other half were defined as the control group and were treated with a vehicle solution. After 75 days of AP treatment, animals were tested on an open-field test to evaluate exploratory, locomotor and anxiety-like behavior. On the 140th day of

treatment, a subset of the animals were anesthetized and their brains were used in order to determine cell count and thickness of the pre-limbic region of the medial prefrontal cortex.

The results indicated that the risperidone group had significantly higher incidences of grooming behaviors in the open field, which the authors assert may be indicative of the rodents experiencing lower levels of stress during the test compared to the control (Spruijt, Van Hooff, & Gispen, 1992). However, no differences were found in cellular counts or thickness of the pre-limbic cortex between treated and non-treated rats (Castellano et al., 2009). Therefore, the authors concluded that chronic administration of risperidone at these dosages did not result in any behavioral changes in healthy animals.

Similarly, De Santis, Lian, Huang & Deng (2016) investigated the long-term effects of aripiprazole, olanzapine and risperidone on rats to see if administration during a critical neurodevelopmental time period led to any differences in locomotion, anxiety, depressive behavior, social interaction and exploration. Drugs were administered in cookie dough from postnatal (PN) day 22 to PN 50 to mimic human childhood and adolescent periods of development. All rats (n=48 male and n=48 female) were randomly assigned to one of the three treatment groups or a vehicle control group.

Medications were given at lower doses in the beginning of the treatment phase and were gradually increased. For the risperidone group, the dosage began at .05 mg/kg three times daily then gradually increased to .3 mg/kg following the first week of treatment. In contrast, the aripiprazole group began treatment at .2 mg/kg and the

olanzapine assigned rats started at .25 mg/kg. Aripiprazole and olanzapine groups were treated with a consistent dosage of 1mg/kg after the acclimation process (one week after beginning treatment).

Behavioral tests were conducted from postnatal day 72 to the 94th day in order to correspond with adulthood in humans. The open-field test was used to assess changes in exploratory behavior and locomotor activity. In addition, anxiety was measured using an elevated plus maze. A social interaction test was used to evaluate whether rats exhibited aggressive behaviors. Lastly, the forced swim test was used to measure depressive-like behaviors.

De Santis et al. (2016) found that male and female rats respond differently to early exposure to AP. Male rats receiving risperidone presented increases in speed, distance and rearing in the open-field test, however, no changes in locomotor activity were found among female rats. Similarly, male rats in the olanzapine group showed an increase in climbing behaviors compared to controls, but a decrease in depressive-like behavior in the forced swim test. Female rats exposed to olanzapine and risperidone spent less time swimming and more time floating than controls and the other drug treated groups.

All three drug treated groups presented anxiolytic-like behaviors on the open field and elevated plus maze tasks. It should be noted that these findings deviate from other research in which no differences in anxiolytic behaviors were found in rats treated with risperidone and haloperidol (Karl et al., 2006), but the discrepancy may be due to

differences in the age of the rats at the time of administration (De Santis et al., 2016). Although both sex groups showed increases in anxiolytic behavior in this study, this effect was stronger in male rats (De Santis et al., 2016). Therefore, males may be more susceptible to behavioral effects of risperidone than their female counterparts. This finding is alarming, given that male children are more likely to be treated with AP in a clinical setting (Domino & Swartz, 2008; Olfson et al., 2012).

Furthermore, Bardgett et al. (2013) conducted a series of studies to determine if differences in locomotor activity, spatial reversal learning and sex differences occurred in 211 young long-evans rats following risperidone treatment. The treatment phase of the experiments began on postnatal day 14 and lasted until postnatal day 42. All rats in the treatment groups received risperidone through subcutaneous daily injections at either 1mg/kg or 3 mg/kg daily. The control group received a daily injection of vehicle solution.

The first experiment observed the effect of risperidone treatment on the locomotor activity of adult rats after long-term administration of the antipsychotic beginning at a young developmental age. Rats were randomly divided into either a vehicle (n=9) 1mg/kg (n=8) or a 3 mg/kg (n=8) risperidone treatment condition. Locomotor activity was measured by placing the rats in polypropylene cages that were located inside a Kinder Scientific Smart Frame photocell activity monitor for 20 minutes a day from postnatal day 49 until day 53. The frequency of photobeam brakes for each rat were then analyzed. The findings for the first study indicated that male rats treated with risperidone showed an increase in locomotor activity as adults one week after the cessation of

treatment. Rats who received risperidone at the higher dose (3mg/kg) were significantly more active than the control and 1mg/kg treated group.

The second study was conducted to determine if these locomotor effects persist into later adulthood. For this study, 27 male rats were randomly assigned to receive risperidone injections at 1mg/kg (n=9), 3mg/kg (n=9) or a vehicle injection (n=9) beginning on postnatal day 14 and ending on postnatal day 42. Locomotor activity was assessed following the cessation of treatment using the photocell-based activity monitor weekly for 7 weeks then again at 9 and 12 months. Although all groups showed a decrease in activity across age, rats that received low and high risperidone doses at a young age presented with greater locomotor activity than controls into adulthood. The degree of locomotor activity change was greater among the group treated with the larger dosage. These findings demonstrated that increases in locomotor activity following risperidone treatment persists into adulthood.

The third trial was done to establish if the presentation of locomotor effects in young rats treated with risperidone were different between sexes. To assess these differences, 60 male rats were separated equally into one of two treatment groups (1mg/kg or 3mg/kg) or the control. Additionally, 56 female rats were assigned to the 3mg/kg treatment group (n=19), the 1mg/kg group (n=18) or the vehicle (n=19) treatment. As with previous experiments, rats were injected with their respected amount of antipsychotic or vehicle on postnatal day 14 through postnatal day 42 and tested for locomotor differences via the photocell-based activity monitor day 49 to 51. An analysis

of the third experiment indicated that male and female rats receiving the higher dosage of risperidone were significantly more active on all test days compared to control rats. Female rats treated with 3mg/kg of risperidone also showed a significant increase in locomotor behavior compared to the low dose treated group.

The final study was intended to decipher if any modifications in reversal learning occur in adult rats following early life risperidone administration. To maintain consistency, 42 male rats were divided into groups of 14 per treatment group and were given the exact same drug treatment during the same time frame as the aforementioned studies. However, this cohort was trained to complete a T-maze maze which is used to assess reversal learning. On postnatal day 56 the rats were given 15g of chow a day. "T" maze training began again on day 63 and all groups were placed in the maze until they ate two bits of food or they could not complete the task in 90 seconds. The habituation process was repeated 3 times daily for 5 full days. By the last day of habituation all rats were able to find the food within the 90 second window. On day 70 acquisition testing began. Rats were placed on the start arm and removed when they chose the goal arm and ate the food. On the first day of testing rats were given 7 trials where the arm the rats were least likely to choose were baited. On the tenth day of assessments, reversal testing began and the site of the baited arm was reversed. The time it took to locate the correct arm was tabulated across trials. No significant differences in spatial reversal learning was found following risperidone treatment.

The summation of these findings suggest that rats may experience increases in locomotor activity following early risperidone treatment (Bardgett et al., 2013; De Santis et al., 2016). These differential effects may last long after the cessation of treatment (Bardgett et al., 2013). However, they may differ depending on dosage of risperidone used (Bardgett et al., 2013) and sex of the rodent (De Santis et al., 2016). Additionally, while Castelleno et al. (2009) found increased grooming in a risperidone treatment group which may be indicative of reduced stress in rodents (Spruijt, Van Hooff, & Gispen, 1992); De Santis et al. (2016) found increased anxiolytic effects in male rats. Therefore, there seems to be a lack of consistency regarding whether risperidone use in young rats leads to anxiolytic-like behavior.

Spatial learning. In addition to locomotor, exploratory and anxiolytic-like affects, there has been some research indicating that spatial learning may also be affected by risperidone treatment on the Morris water maze task (Mandell, Unis & Sackett, 2011; Skarsfelt, 1996; Terry et al., 2003). However, only a few studies have been conducted in order to determine if risperidone has any effect on a rodent's ability to perform on the Morris water maze. To date, no studies exists looking at the effect of risperidone following early treatment.

A drug comparison study was conducted by Skarsfelt (1996) in order to determine what effects olanzapine, seroquel, ziprasidone, clozapine, sertindole, haloperidol and risperidone had on place navigation of 3-month old male wistar rats using the Morris water maze. All groups (N=8-10) were given injections at various dosages. The

risperidone treated rats were placed in one of four dosage conditions (.19 mg/kg, .39 µg/kg, .76 µg/kg, 1.5 µg/kg) and were administered via injection 30 minutes prior to each daily testing session. The control group was injected with a saline solution and participated in all behavioral experiments. Prior to each trial, rats were placed on a hidden platform for 15 seconds. For four consecutive days, rats completed three trials in which the start position was randomized so that all possible locations were used. If the rat was unable to complete the task within 60 seconds it was gently guided toward the platform.

For each trial, escape latency and swim speed were measured. Escape latency was thought to be a good measurement of spatial memory, while swimming speed gave the researchers an indication of motor function changes that may be occurring in response to medications (Lindner & Schakkert, 1988; Von Lubitz, Paul, Bartus, & Jacobson, 1993). Skarsfelt (1996) found that sertindole and seroquel did not significantly affect Morris water maze performance. The clozapine group showed impaired performance during the first two days of testing. However, no significant differences were found in the clozapine group during the last two days of assessments compared to control. Conversely, ziprasidone and olanzapine groups showed significant deficits in their abilities to maneuver the Morris water maze during all four trials.

Likewise, risperidone and haloperidol experimental groups showed marked impairments in their ability to find the platform at the higher dosages (.76µg/kg, 1.5 µg/kg). At the lower dosages (.19 mg/kg, .39 µg/kg) a decrease in swim speed was found in the risperidone treated rats. Together, these results show that drugs such as risperidone,

haloperidol, ziprasidone, and olanzapine may lead to spatial memory impairments of performance in rodents and these deficits may be dose dependent.

Terry et al. (2003) conducted a similar study in order to determine the effect of chronic exposure to haloperidol (2mg/kg/day), risperidone (2.5mg/kg/day) and clozapine (20mg/kg/day) on spatial learning and cholinergic markers. Drugs were administered in water bottles to male rats weighing 225 to 250g (N=30). Rats assigned to the control condition were given a daily dose of citric acid (N=8), acetic acid (N=8) or tap water (N=12) to ensure changes to the rats' behavior did not occur due to vehicle administration. Following a 45-day period of treatment and a 4-day washout, 15 animals in each group were assessed using the Morris water maze. This task was chosen because performance on the task necessitates an intact cholinergic system and employs the hippocampus and other medial temporal lobe structures known to be important components for human cognition (McNamara & Skelton, 1993).

Following behavioral testing, brains were removed and number of choline acetyltransferase (ChAT) immunopositive cells in the cortical and subcortical areas were measured using an indirect immunofluorescence method (Terry et al., 2003). After 90 days of treatment, the other subset of rats were assessed on the Morris water maze after a four day wash out period. Brains were then analyzed to determine if long-term treatment groups exhibited any differences in the number of ChAT cells compared to the 45-day treatment and control groups.

Results indicated that haloperidol significantly impaired spatial learning performance after 90 days of treatment compared to controls and other drug treated groups. Cholinergic cell counts showed reduced ChAT staining after 45 days of treatment. Clozapine treated groups did not show any significant changes even after 90 days of exposure to the medication. Conversely, risperidone slightly improved cognitive task performance and swim speed. Despite these findings, the risperidone treatment group showed a significant reduction in the number of ChAT stained cells following 90 days of treatment. This finding indicates not only that different drugs may have differential long-term effects on cholinergic markers, but also that long-term treatment of haloperidol and risperidone may lead to neurochemical alterations in the brain that could potentiate later cognitive impairments.

Therefore, some evidence exists indicating that risperidone may adversely affect Morris Water Maze latency in a dose dependent manner (Skarsfelt, 1996). However, treatment has also been found to enhance performance in the Morris water maze compared to other treatment groups (Terry et al., 2003). In both studies, the researchers used older rats when assessing how risperidone affects spatial abilities (Skarsfelt, 1996; Terry et al., 2003). Additional research needs to be conducted in order to determine risperidone's effect on rats following early administration. Furthermore, reduced ChAT staining was found in the hippocampus and caudate-putamen and these areas are important for cognitive functioning (Terry et al., 2003). Consequently, longer-term

studies need to be conducted to determine if chronic treatment of risperidone elicits behavioral impairments in spatial learning and memory function.

Current Study

Currently, AP are being prescribed to children at unprecedented rates (Hutchings et al., 2013; Masi et al., 2015; Qui et al., 2014). However, very little research has been conducted on young populations due to ethical and environmental limitations (Bardgett, et al., 2013; Menard et al., 2014). Preclinical studies using rodent models are important to assess potential consequences of such treatments (Bardgett et al., 2013; Pandina et al., 2007).

Even though risperidone is the most commonly used AP in the pediatric population (Bardgett et al., 2013; Memarzia et al., 2016), the majority of pre-clinical research regarding the possible effects of risperidone are comprised of studies using adult rodents (Andersen & Navalta, 2011; Castellano et al., 2009; Menard et al., 2014;). Accumulating evidence is beginning to suggest that, juveniles may be more prone to the negative developmental effects of AP use (Andersen & Navalta, 2011; Bardgett et al., 2013; Findling et al., 2010; Frost & Cadet, 2000; Levitt et al., 1997; Lian et al., 2016; Mandell et al., 2011; Moran-Gates et al., 2007; Qiao, et al., 2013; Tarazi & Baldessarini, 2000; Vinish et al., 2013). Therefore, intervention during late childhood to early adolescence (defined as 35-60 days in rats; developmentally similar to 10-19 human years) (Andersen et al., 2000) may induce permanent changes to neural circuitry which

may sequentially lead to longer-term behavioral changes (Castellano, et al., 2009; Frost et al., 2009; Lian et al., 2016; Mandell et al., 2011; Menard et al., 2014; Singh & Chang, 2012).

The few risperidone studies that have been conducted using young rodents have indicated that behavior may be impaired on certain behavioral assessments following persistent risperidone treatment (Bardgett et al., 2013; Castellano et al., 2009; De Santis et al., 2016; Skarsfelt, 1996; Terry et al., 2003). These behavioral consequences seem to vary depending on route of administration, dosage administered and length of AP treatment (Castellano et al., 2009; De Santis et al., 2016; Terry et al., 2003). The majority of the aforementioned studies placed their emphasis on determining the neurobehavioral effects of risperidone treatment in rats following injection or oral consumption (Castello et al., 2009; Bardgett et al., 2013; De Santis et al., 2016; Skarsfelt, 1996; Terry et al., 2003).

These methodologies can create confounding variability which may limit the validity of the study (Bardgett et al., 2013; Kapur, VanderSpeck, Brownlee, & Nobrega, 2003). For example, long-term use of injections may cause a great deal of stress to rats which may affect behavioral outcome (Terry et al., 2007a). Oral consumption may lead to differences between rats in how much medication they are consuming daily (D'Souza, Faraj, & DeLuca, 2013). Additionally, single-daily-dosing strategies often employed in these types of administration, often produce variations in blood drug levels and brain receptor occupancies that are not typical to what would occur in a clinical setting

(Bardgett et al., 2013). Therefore, continuous drug infusion tactics are needed to reduce variability and create more clinically relevant studies (Bardgett et al., 2013; Karl et al., 2006; Su et al., 2011; Tarazi, Zhang & Baldessarini, 2001). To date, no risperidone studies have been conducted on young rats using this route of administration.

Additionally, most studies focus on behavioral effects following high dosages of risperidone (Memarzia et al., 2016). Little is known about the effects of low dose risperidone on behavior. This is an important gap to address, because there is evidence that young rats may have a lower threshold for neurobehavioral effects, but little is known regarding when that threshold begins.

To date, few studies have been conducted to examine the neurobehavioral effects of chronic administration of risperidone on a juvenile population (Bardgett, et al., 2013; Curtis et al., 2005; Masi et al., 2015). This gap in the literature is of great clinical importance because children who begin medications at a young age are more likely to remain on them for long periods of time and are less likely to discontinue medication in adulthood (Memarzia et al., 2016). Therefore, the purpose of the current study was to examine whether a 6-week low dose treatment of risperidone would affect the performance of juvenile male rats on a variety of behavioral tasks designed to emulate locomotor, exploratory, anxiety-like, spatial recognition, spatial memory and spontaneous alternation behavioral performance.

CHAPTER III

METHOD

Animals

In accordance with the National Institute of Health guidelines for care and use of animals, this research was approved by the Animal Care and Use Committee at West Texas A&M University. Since male children are more likely to be given AP in a clinical setting (Domino & Swartz, 2008; Olfson et al., 2012) twenty-four juvenile (1-month old) male Sprague Dawley rats were obtained from Charles River. Animals were multiply housed (3 per cage) and maintained in a temperature-and light-controlled environment with a 12h light, and 12 h dark cycle. All rats were given food and water *ad libitum*.

Pellet Implantation

Following a one-week adjustment to the facility, rats were randomly assigned to either a risperidone pellet (N=12) or placebo pellet (N=12) condition. At 5 weeks of age, rats received subcutaneous implants (dorsal neck) of either risperidone (2.5 mg; 60-day time release; Innovative Research of American Inc.) or a placebo pellet (control surgery). risperidone treated rats received an equivalent of .04 mg/day. For this surgery, rats were

anesthetized using isoflurane (4% induction, 2% maintenance in 70% N_2O and 30% O_2 mixture.

This age was chosen because post-natal day 34-47 is thought to encompass the peri-adolescent to adolescent time frame which is a crucial time for brain development (Blakemore & Choudhury, 2006; Piontkewitz, Arad, & Weiner, 2011, Piontkewit et al., 2012). The dosage used in this study is comparable to that of other rodent studies (Farrelly et al., 2014; Piontkewitz, Arand & Weiner, 2011; Piontkewitz et al., 2012; Richtand et al., 2006) and is considered to be a low dosage for this age group (Farrelly et al., 2014; Grayson, Idris & Neill, 2007; Wiley, 2008). The 42-day treatment period before beginning the behavioral tests, is a considerably longer treatment duration than many of the available long-term studies (Bardgett et al., 2013; De Santis et al. 2016).

Behavioral Testing

Six weeks following pellet implantation, rats underwent behavioral testing. Rodents were roughly 11 weeks old (PN 77) at the time of behavioral testing, which is thought to be indicative of early adulthood (Qiao et al., 2014; De Santis et al. 2016; Schneider, 2013; Stevens et al., 2016).

Open-field. The open-field task assesses locomotion, exploration, and anxiety-like behavior (Prut & Belzung, 2003). Testing was conducted in a square black box (57.6 cm x 57.6 cm, 38 cm high). The floor of the open field box was divided into nine equal squares. Rats were placed in the open field for six minutes. The behaviors measured

included locomotion, assessed by the number of grid crosses and defined as all four paws crossing a grid line. Rearing behavior, a measure of exploration was defined as lifting of the upper body and forepaws off the ground. Anxiety-like behavior was measured as the amount of time (sec) the rats had all four paws in the center square. However, the interpretation of center behavior as being indicative of anxiety-like emotionality has recently been called into question (Emaceur, 2014). To remain consistent with the available literature (Castellano, 2009; Durand et al., 1999; Hiroi, McDevitt, & Neumaier, 2006; Prut & Belzung, 2003; Walsh & Cummins, 1976), the current study considered a decrease in the amount of time spent in the center square to be evidence of increased anxiety-like behavior.

Object and Spatial Recognition. One day following open-field testing, a rodent's visual memory was assessed using the object recognition task (Jiwa, Garrard, & Hainsworth, 2010; Walsh & Cummins, 1976). This task has an advantage over other tasks because it does not require food and water deprivation or learning of response-reward associations (Dere, Huston, & Silva, 2007). To measure object recognition, rats were placed in the open field with two identical objects and the amount of time (in seconds) spent exploring each object was recorded for 3 minutes (Trial 1). Exploration was operationally defined as rearing towards or on an object, touching object with their paws, sniffing or looking at the object from a distance less than 2 cm. After each trial, alcohol was used to clean the objects in the open field in order to decrease the possibility of olfactory cues affecting behavioral observation (Grayson et al., 2007). Following a

retention interval of 1 h, rats were returned to the open field for 3 minutes (Trial 2) with a familiar object and a new novel object. Time spent exploring each object was then recorded. A preference score was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects multiplied by 100. A preference score of 50% represented chance levels and a higher score indicated intact object recognition.

To measure spatial recognition, rodents were positioned in the open-field with two identical objects and the amount of time (in seconds) spent exploring each object was recorded for 3 minutes (Trial 1). Exploration was operationally defined as behaviors such as rearing towards or on an object, touching object with their paws, sniffing or looking at the object from a distance less than 2 cm. Following a 30 minute delay, rats were again placed in the open-field with the same objects (Trial 2). However, one of the objects was moved to a new position and exploratory behavior was again recorded. A preference score was obtained by dividing the time spent exploring the displaced object by the amount of time they spent exploring both objects multiplied by 100.

Morris Water Maze (MWM). Following both Object and Spatial Recognition testing, rats were tested on the MWM to assess spatial memory. This task relies heavily on hippocampal functioning and is frequently used to test spatial learning and memory (Morris, Garrud, Rawlins & O'Keefe, 1982; Terry et al., 2003). MWM testing was performed in a blue circular pool 182.88 cm in diameter and 76.2 cm in height which was filled with water (21 $\pm$1^{o} C). A clear Plexiglas platform was submerged 2 cm under the

water. Rats were given 4 trials per day for 4 consecutive days to find a hidden platform. Rodents were randomly placed in one of four quadrants (designated north, south, east and west) and given 90 seconds to find the hidden platform which remained in the Northeast quadrant across all trials and days. If the rat was unsuccessful within the allotted time frame it was guided to the platform and allowed a 15 second rest period. Latency (sec) to find the platform was recorded and averaged across each training session.

Y-Maze. Spontaneous alternation testing took place using a Y-maze, and was performed as described by Rahmati et al. (2017) and De Butte-Smith, Etgen, Gullinello, & Zukin (2009). The Y-maze assesses for behavioral differences in the rat's natural tendency to alternate in a nonreinforced manner between successive arm choices (Hughes, 2004). The apparatus was made up of three equivalently spaced arms (55.9cm long x 25.4cm high). Each rat began their trial by being positioned at the same start point within the maze and given 10 minutes to explore. Number of arms and order in which the rats entered each arm was recorded. If the rat completed three different sequential arm visits, a spontaneous alternation pattern was recorded. A same arm return was recorded if the rat returned to the same arm following a previous exploration of that arm. If the rat explored two arms consecutively then proceeded back to the first one, the behavior was designated as an alternate arm return. A percent alternation score was calculated by dividing the number of spontaneous alternations made by each rat by the total number of triplets then multiplying that quotient by 100.

Research Design

The basic design of the study was a between groups comparison for the two drug conditions (risperidone and sham) on the open field, object recognition, spatial recognition and Y-maze tasks. In addition to the between groups design (risperidone, sham), the Morris Water Maze task also included the within subject's comparison (day) in order to assess the interaction between day and group.

CHAPTER IV

RESULTS

Open-Field

To assess whether risperidone treatment produces any global behavioral changes, locomotion, exploration, and anxiety-like behavior was measured using an open field. A MANOVA was conducted in order to compare number of grid crosses, rears, and time spent in the center between groups. Risperidone did not appear to affect locomotor activity [$F(1,22) = 4.13$, $p=.054$] as illustrated in Figure 1A. Additionally, no differences were detected in the exploration or anxiety-like behavior (Figure 1B, C). Specifically, both groups did not differ in the number of rears [$F(1,22)=.78$, $p=.38$] and spent a similar amount of time in the center of the open field [$F(1,22)=.00$, $p=.98$].

Object and Spatial Recognition

To assess whether risperidone would affect visual memory, rats were tested on the object recognition task. As illustrated in Figure 2, no differences were observed in risperidone treated rats on the component of visual memory performance [$F(1,21) =.02$, $p=.87$]. All rats demonstrated above chance preference scores. For the spatial recognition task, one rat was excluded from the risperidone group due to insufficient exploration of the objects during the training session.

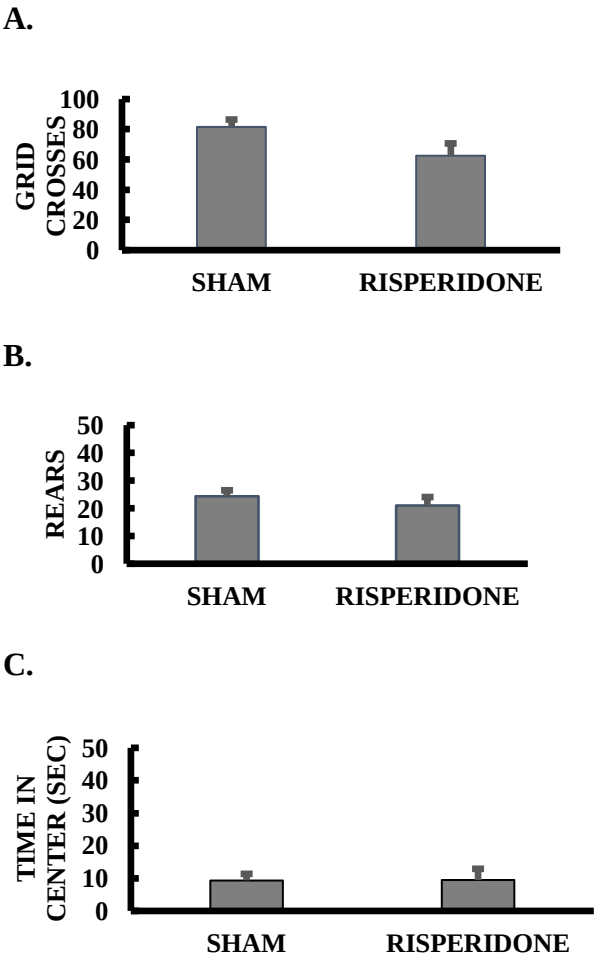

Figure 1. No significant differences were observed in the risperidone treated rats on the open field task. [A] Locomotor activity was measured as the number of grid crosses. Data are presented as X ± SEM. Risperidone treated rats showed a trend towards fewer grid crosses but this missed significance (p =.054). [B] Exploration was measured as the number of rears. [C] Anxiety-like behavior was measured by the amount of time (in seconds) the rat spent in the center of the open field.

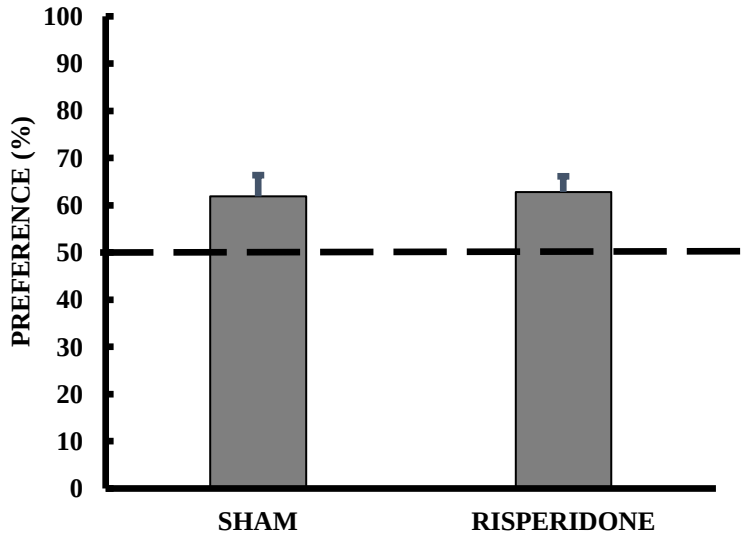

Figure 2. No significant differences were detected in visual memory performance on the object recognition task. Data are reported as preference scores (novel object exploration/total object exploration, %, $X \pm$ SEM) for 3-min trials. A retention interval of 1 hour was given between training and test sessions. Line at 50% represents equal exploration of both objects (chance-performance).

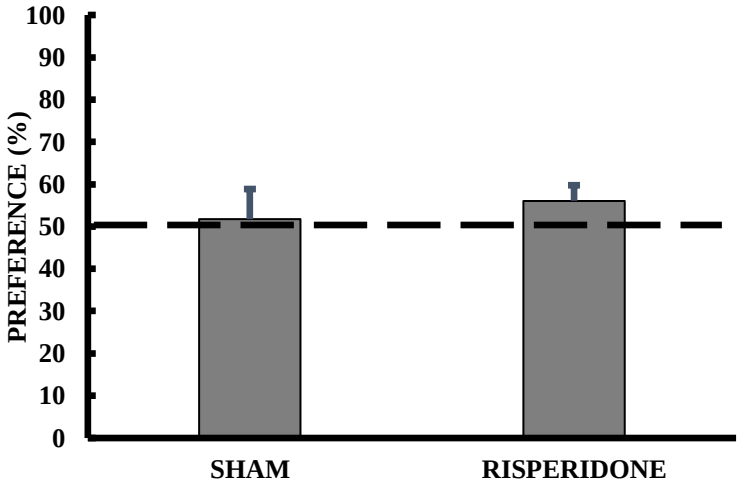

Figure 3. No significant differences in spatial recognition performances were observed on the spatial recognition task. The data are reported as preference scores (displaced object exploration/total object exploration, , %, $X \pm$ SEM*)* for 3-min trials. A retention interval of 30 minutes was given between training and test sessions. Black dashed line at 50% represents equal exploration of both objects (chance performance).

To assess for differences in spatial recognition, a one-way ANOVA was initially conducted. It was determined that the assumptions of normality and homogeneity of variance were violated. To compensate for these violations, a Mann-Whitney U non-parametric test was performed to evaluate if risperidone treated rats exhibited differential exploratory behavior towards a displaced object compared to controls. As illustrated in Figure 3, no significant difference was found between risperidone treated and non-treated rats in regards to spatial recognition performance. Both groups exhibited above chance preference for the displaced object ($z = -.49$, $p > .05$).

Morris Water Maze

To assess the effect of risperidone on hippocampal-dependent spatial reference memory, groups were tested in the MWM. A mixed factorial ANOVA with group (risperidone and control) as the between subjects factor and day (1-4) as the within subjects factor revealed no significant group differences [$F(1,22) = .73$, $p = .40$] as illustrated in Figure 4. A significant effect of day revealed that all rats exhibited shorter latencies across days [$F(1,22) = 112.82$, $p < .001$]. Post-hoc analyses using paired-sample t-tests were adjusted using Sidak alpha adjustment for multiple comparisons. The t-tests revealed that both risperidone and placebo treated rats spent significantly more time searching for the platform on day 1 compared to day 2, 3, and 4 ($p < .01$). Additionally, all

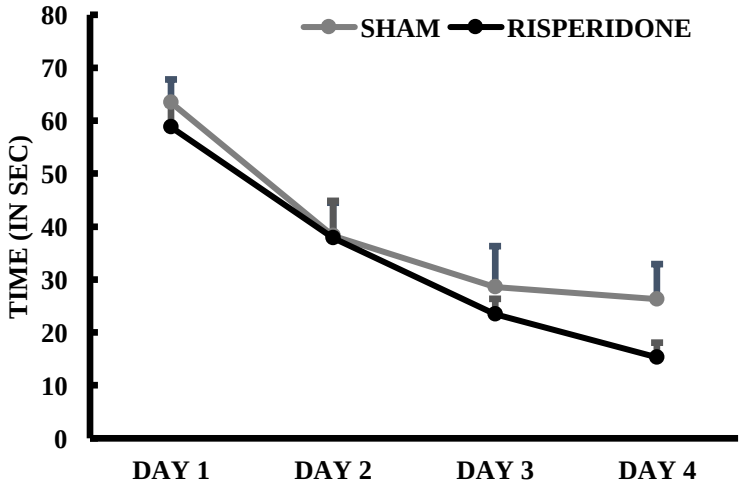

Figure 4. The risperidone and sham groups did not demonstrate differences in spatial memory performance on the Morris Water Maze task. Data represents the mean escape latency times exhibited by both groups for each day (1-4). Error bars represent *X*± S.E.M.

rats exhibited longer latencies on Day 2 compared to Day 3 ($p<.05$) and 4 ($p<.01$). No difference was found between latency time on Day 3 and 4 ($p>.05$).

Y-maze

After an initial one-way ANOVA reported violations of homogeneity of variance and normality, a Mann-Whitney U non-parametric test was used to assess for differences in spontaneous alternation pattern (SAP), alternate arm return (AAR) and same arm return (SAR) behavior between risperidone and placebo treated rats. No significant differences were observed between the risperidone and sham group on percentages of spontaneous alternation ($z = -1.88$, $p > .05$) or alternate arm returns ($z = - .14$, $p > .05$) behaviors. However, risperidone treated rats displayed significantly more same arm returns (went back into the same arm they had previously been in) compared to placebo treated rats ($z = -3.01$, $p < .003$) as illustrated in Figure 5.

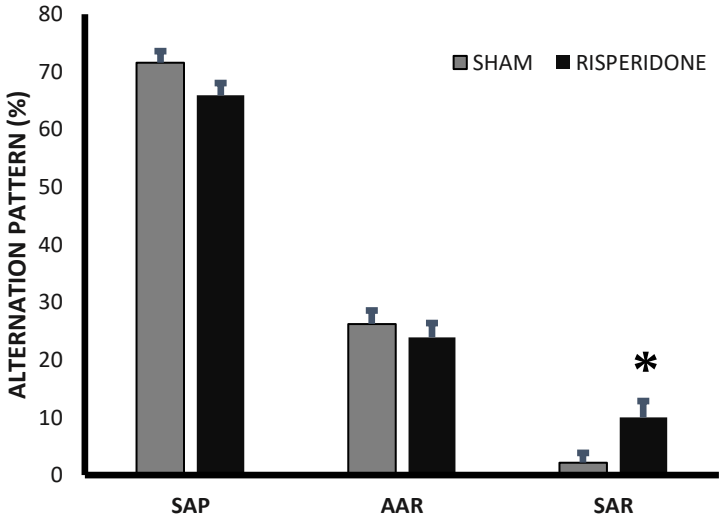

Figure 5. Risperidone treated rats did not significantly demonstrate spontaneous alternation or alternate arm return impairment on the Y-maze. Risperidone treated rats did show significantly more same arm returns compared to placebo rats. Data are reported as alternation scores (condition alternation pattern/total alternation pattern, %) following a 10-min session. Error bars represent $X\pm$ S.E.M. * represents $p<.05$.

CHAPTER V

DISCUSSION

Risperidone treated rats did not show significant changes in locomotor activity, exploration, or anxiety-like behavior

The open field task was used to assess for differences in locomotor, exploratory and anxiety-like activity; and was also used to identify rats displaying sickness behaviors in order to eliminate those with physical limitations from the remainder of the behavioral assessments (Seibenhener & Wooten, 2015; Walsh & Cummins, 1976). The open field task yielded no evident sickness-like behaviors or significant differences between the risperidone treated and placebo rats on locomotor, exploration, or anxiety-like behavior. This finding is consistent with Nowakowska et al. (1999) who did not discover any differences in the locomotor activity of adult rodents after a two-week administration of risperidone (.15 mg/kg). Additionally, Castellano et al. (2009) also did not find a significant difference in locomotor behavior (grid crosses) in juvenile rats treated chronically (120 days) with risperidone.

Although not statistically significant, the data in current study is suggestive of a trend towards decreased locomotion among the risperidone treated animals. Previous studies have found that risperidone may lead to decreases in motor activity, particularly at

dosages below the extrapyramidal (EPS) side effect threshold in adult rodents (Broderick, Rahni & Zhou, 2003; Hutchings et al., 2013; Karl et al., 2006). Additionally, low-dose risperidone injections (45µg/kg or 85µg/kg) was found to prevent an increase in activity among psychosis model rats (used amphetamine) following hippocampal lesions (Richtand et al., 2006). Therefore, further risperidone studies using varying dosages of risperidone are needed to better understand the relationship between dosage and locomotor activity.

The current study also did not reveal any significant differences in exploratory behavior between risperidone and placebo treated rats. This finding is in accordance with a study by Castellano et al. (2009) who also did not observe any significant differences in exploratory behavior on the open field following early chronic administration (5-weeks to 120 days) of risperidone (1mg/kg/day).

In further agreement with Castellano et al. (2009) and Karl et al. (2006) no anxiety-like (time spent in the center) differences were found in the current study between the risperidone treatment group and the control on the open field task. Conversely, De Santis et al. (2016) found anxiety-like effects on the radial arm maze and open field following early (PN 22-50) exposure to risperidone in adult rats (testing began on PN 72). It could be that the discrepancies occurring between the current findings on the open field and other similar studies may be due to inconsistencies in the dosages used, sex of subjects, tasks being administered, and age of the rats upon administration (Bardgett et al., 2013; Castellano et al., 2009; De Santis et al., 2016; Terry et al., 2003).

Risperidone treated rats did not display impairments in visual or spatial recognition on the object or spatial recognition tasks

Object and spatial recognition tasks are based on a rodent's natural tendency to prefer novel over familiar objects (Dere et al., 2007). Since rats typically approach and investigate novel objects more readily, it is assumed that the initial explorative episodes (trial 1) on the object recognition and spatial recognition tasks leave an enduring memory trace regarding which objects have been explored and where the objects were encountered. Therefore, preference towards a novel object or displaced object can be quantified in order to assess for differences in visual and spatial recognition memory (Aggleton, 1985; Dere, et al., 2007; Jiwa, Garrard, & Hainsworth, 2010). In the current study, no significant differences were found between the risperidone and placebo conditions. To date, no other studies have assessed the neurobehavioral effects of early life exposure to risperidone using these tasks.

However, Terry et al. (2007a) evaluated the effect of risperidone (2.5 mg/kg/day) on object recognition, using three different delay times (1 minute, 15 minutes and 60 minutes) in adult rodents after 8-14 days and 31-38 days of treatment. Consistent with the current study, no significant effect was found in the treatment group following the one-hour delay at either time-period. A decrease in retention (time spent exploring novel object) was found in the risperidone treatment group compared to vehicle after a one-minute delay on days 31-38 of treatment. Therefore, differences in delay times may generate differences

in task performance in risperidone treated rats. As such, future studies may expand upon the current findings by using multiple delay times on these tasks.

Additionally, the current study yielded a control group with a preference score above chance performance for both tasks. However, it should be noted that the preference for the control group on the spatial recognition task narrowly exceeded chance performance. Jablonski, Schreiber, Westbrook, Brennan & Stanton (2013) also found that exploration was much lower in young rats on the spatial recognition task compared to the object recognition task in heathy rats. The inconsistency occurring within the control groups of these studies calls into question whether spatial recognition may be a more difficult task for this age group attain. If this is the case, perhaps future studies should require longer exploration times (more than 3 minutes) in order to acquire an adequate initial investigation of the objects.

Risperidone treated rats did not show differences in spatial memory on the MWM

The Morris water maze task (MWM), requires a higher level of spatial information processing than the spatial recognition task (Hok et al., 2016). Inside the maze, the platform offers no cues, so the rats have to rely on the environments extra-maze cues to map out the spatial relationship between oneself and the hidden platform to escape the water in a timely manner (Morris, 1984; Hok, Poucet, Duvelle, Save, & Sargolini, 2016; Schoenfeld, Schiffelholz, Beyer, Leplow, & Foreman, 2017). For the current study, risperidone and placebo treated rats were able to learn the location of the

escape platform across training sessions as evidenced by shorter latency times across days.

Also, the sham and low dose risperidone treated rats did not demonstrate any significant differences in latency times in the MWM. Similarly, Skarsfeldt (1996) assessed adult rat performance on the MWM task after administering one of four risperidone injection treatments (.19mg/kg, .39 µg/kg, .76µg/kg, 1.5 µg/kg) 30 minutes prior to the first test trial. No significant differences were found at the two lower dosages. Conversely, at higher dosages (.76µg/kg and 1.5µg/kg) a significant impairment in performance on the Morris water maze task was observed. This finding (Skarsfeldt, 1996) along with several others that have been discussed in this review have indicated that risperidone may yield dose dependent effects on behavior (Bardgett et al., 2013; Grayson et al., 2007; Moran-Gates et al., 2007).

One possibility for these findings, is that greater dosages of AP have been largely associated with extrapyramidal syndrome (EPS) (Parkinson-like symptoms) and these effects may lead to difficulties completing assessments requiring physical movement (Casey, 1996; Ferreia et al, 2016). These side-effects may introduce confounding variability in behavioral studies with AP. Therefore, one strength of the current study is that the dosage administered was low, which enabled the rodents to complete the behavioral tasks without the accompanying loss of motor-control that may occur at greater dosages.

In the current study, no significant differences were observed between the risperidone and sham groups on the MWM at 45 days of treatment. In accordance with our finding, Terry et al (2007a) examined MWM task performance in adult rats at 5 different time points (8-14, 22-28, 30-45, 84-90, & 174-180) following oral risperidone (2.5mg/kg) treatment. The groups did not differ until treatment days 84-90. A second study was conducted by Terry and colleges (2007b) and also yielded no significant differences in middle aged rats following a 45-day treatment period.

Interestingly, in the Terry et al., (2007a) study that was previously discussed, a significant decrease in latency time was observed on treatment days 83-90, while an increase in water maze impairments occurred during treatment days 174-180 (Terry et al., 2007a). These findings seem to indicate that behavioral effects on the Morris water maze may be time dependent (Terry et al., 2007a; Terry et al., 2007b; Terry et al., 2003; Terry & Mahadik, 2006). Therefore, future studies should be conducted in order to determine if early-life exposure to risperidone generates differences in spatial memory on the MWM after longer treatment periods.

Risperidone treated rats did not demonstrate differences in SAP or AAR but did show increases in SAR patterns on the Y-maze

The Y-maze was used to assess for differences in spontaneous alternation behaviors (Lalonde, 2002). Rats have a natural tendency to alternate between arm choices (Bak, Pyeon, Seok, & Choi, 2016; Myhrer, 2002; Richman, Kim & Dember, 1986; Yadin,

Friedman & Bridger, 1991) and will typically alternate at levels significantly above chance (Lalonde, 2002). One of the appeals for conducting this task was that it is a simple and reliable way to assess for differences in typical rat behavior that does not require them to be exposed to stressful stimuli or require extensive training of reward-response associations. (Hughes, 2004; Richman, Kim & Dember, 1986). In the current study, no significant differences were demonstrated between the risperidone pellet condition and the control in SAP or AAR. This finding is convergent with Delotterie et al. (2010) who did not find any differences on the y-maze in genetically altered (STOP) mice after 4 weeks of vehicle or risperidone treatment (.1mg/kg/day or .3mg/kg/day).

Although behavior on the SAP and AAR components of the y-maze were intact, the risperidone group displayed a significantly greater number of SAR's than the controls. This refers to the tendency of the risperidone treated rats to re-enter an arm they had immediately exited. To date, no other risperidone study using healthy rats has been conducted using the y-maze task.

However, Karl et al (2006) used a similar task called the cross maze (has eight arms) and found that adult rats exposed to risperidone after a four-week (2.5 mg/kg/day) treatment period displayed impairments in the ability to recall which arm they had previously entered. Several authors have claimed that these lapses in recall on the y-maze and cross maze can be attributed to a deficit in working memory (Hidaka, Suemaru & Araki, 2010; Karl et al, 2006; Lolonde, 2002; Sarter, Bodewitz & Stephens, 1988; Wright & Conrad, 2005). Taken together with our finding, although intriguing, more research

using more standard working memory tasks such as the Radial Arm Maze should be explored (Hughes, 2004).

The mechanisms behind why risperidone may affect working memory in rodents are not well understood (McGurk et al. 2004; Reilly et al., 2007). However, several neurochemical studies have discussed the probability of antipsychotics leading to alterations to the dopaminergic and serotonergic system within the dorsolateral prefrontal cortex (Abi-Dargham et al., 2002; Camchong et al., 2006; Choi et al., 2010; Hirvonen et al., 2006; Lian et al.,2016; Lidow, Elsworth, & Goldman-Rakic, 1997; Moran-Gates et al., 2007; Myhrer, 2003; Von Huben et al., 2006) and hippocampus (Moran-Gates et al., 2007). Thus, alterations to neurotransmitter systems within these structural areas may be contributory to the current deficit in working memory. Therefore, future studies need to be conducted to further clarify how early risperidone treatment contributes to these specific areas.

Conclusion

In recent decades, there has been a substantial increase in the number of children prescribed AP's for the treatment of several psychiatric conditions (Masi et al., 2015, Olfson et al., 2012). Hence, pre-clinical rodent studies are important to elucidate the potential neurobehavioral consequences of these drugs. Several rodent studies have shown that juvenile rats may be more susceptible to neurodevelopmental alterations in the brain following early-life exposure to AP treatment (Choi, et al., 2009; Lian et al., 2016; Milstein et al., 2013; Moran-Gates et al., 2007; Piontkewitz et al., 2011; Qiao, et

al., 2013; Schneider, 2013) and which may induce lifelong behavior changes in adulthood (Bardgett, et al., 2013; Lian et al., 2016).

However, few studies have been conducted to evaluate whether early chronic risperidone treatment leads to later behavioral impairments, especially regarding the novel atypical class of AP medications such as risperidone (Bardgett, et al., 2013; Curtis et al., 2005). This lack of literature has resulted in a great deal of controversy among clinicians and researchers regarding whether or not it is safe to give AP to children (Memarzia et al., 2016; Pandina et al., 2007). Therefore, the current study was conducted in order to begin the process of generating the desperately needed studies that will help professionals to better assess if it is safe to give these medications to this potentially more vulnerable population.

The current study did not find any behavioral differences between the risperidone and sham pellet condition on the open field, object recognition, spatial recognition or MWM task after a 6-week low dose administration of risperidone. Interestingly, the study also did not demonstrate differences on SAP and AAR but did find that the risperidone treated rats had significantly more SAR behaviors. Several authors have concluded that this lack of recall may be thought of as an indicator of a deficit in working memory (Hidaka et al., 2010; Karl et al, 2006; Lolonde, 2002; Wright & Conrad, 2005; Sarters et al., 1988). Since no locomotor or exploratory effects were evident in any of the other tasks, this theory that working memory may be affected by early risperidone treatment does seem to hold some merit. However, since no other studies have been conducted

looking at the effect of early risperidone treatment using the y-maze or other spatial working memory tasks, more work needs to be done to better understand the possible meaning of these findings.

From the results obtained in this study, it may be concluded that risperidone at the current dosage (.04 mg/day) and treatment duration (6 weeks) does not severely impact rodent behavior in adulthood. This finding is in accordance with other previous studies assessing the safety of risperidone (Castellano et al., 2009; Keith, 2009; Kissling, Glue, Medori, Simpson, 2007; Lindstrom, Eberhard, & Levander, 2007). While, these results have important clinical and preclinical implications, more studies will need to be done in order to ascertain whether or not they generalize to the human population.

In several similar studies, a dose-per-day approach was employed (Choi et al., 2009, 2010, Mandell et al., 2011; Moran-Gates et al., 2007; Soiza-Reilly & Azcurra, 2009). A limitation to this dosing strategy is that most AP have a half-life of a few hours in rats (Kapur et al., 2003). Therefore, single day administration of medications may lead to fluctuations in the blood drug levels within the brain receptor occupancy levels which are not typically found in human's receiving AP treatment. Consequently, one strength of the current study is that a continuous release pellet was subcutaneously implanted to insure the rat was receiving a continuous dosage daily throughout the study (Bargett et al 2013; Karl et al., 2006)

Additionally, the current study also utilized low dosage of risperidone, in order to provide information about the threshold of drug symptoms and reduce the likelihood of

losing animals. Despite the conservative nature of our dosage, it is still well within the range of the average clinical dosage often given to youths (Aravagiri & Marder, 2002; Farrelly, et al., 2014; Heykants, et al., 1994; Newcomer, 2005; Van Beijsterveldt et al., 1994). However, since several researchers have reported dose-dependent effects (Bardgett et al., 2013; Grayson et al., 2007; Moran-Gates et al., 2007; Skarsfeldt, 1996), it is important for future studies to determine if similar results occur following larger dosages of risperidone. Another important direction for future studies to investigate is the effect of poly-drug therapy. It has been estimated that 40% of the children that are prescribed AP are using more than one type of drug (example: antipsychotic and mood stabilizer) (Dusetzina et al., 2012). Therefore, studies focusing on behavioral consequences of early life risperidone treatment in conjunction with other relevant medications are desperately needed, to ensure that these AP treatments will be administered to children in an informed manner.

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