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Consequences of In Utero Exposure to Alcohol with a Focus on Behavioral Outcomes

Brittany N. Bamberg
University of South Dakota

CONSEQUENCES OF *IN UTERO* EXPOSURE TO ALCOHOL WITH A FOCUS ON BEHAVIORAL OUTCOMES

By

Brittany Bamberg

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The members of the Committee appointed to examine
the thesis of Brittany Bamberg find it
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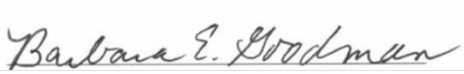

Barbara E. Goodman, Ph.D.

Angela Landeen, MS, CHES, CPH

ABSTRACT

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With a Focus on Behavioral Outcomes

Brittany Bamberg

Thesis Director: Dr. William Mayhan, Ph.D.

In utero exposure to alcohol can result in fetal alcohol spectrum disorder (FASD), which encompasses a range of developmental disorders. The prevalence of FASD has been estimated to impact approximately 40,000 children in the U.S. each year. These individuals are at a greater risk for cognitive dysfunction, dementia, and seizures. The goal of this study was to determine the influence of *in utero* exposure to alcohol on cognition and behavior in adolescent and adult rats. Pregnant rats were fed an alcohol diet (3% alcohol) throughout their gestational period or control diet (no alcohol). Pups were weaned and pair-housed within sex and treatment groups, under reverse 12-hour light 12-hour dark cycle and allowed to reach 4 weeks of age prior to behavioral testing. Cognitive and social behavior was evaluated using a Novel Object Recognition task (NOR), a Spatial T-maze test, and a Social Interaction test (SI). Upon completion of adolescent testing, each pair was allowed to reach adulthood undisturbed, before repeating the tests at 14 weeks of age. Our results suggest that rats exposed to alcohol *in utero* have a significant impairment in working memory (short-term), suggesting cognitive impairment, however, only sex and age differences were revealed in spatial/long-term memory and SI. Taken together, rats exposed to *in utero* alcohol exhibit behavioral deficits indicative of impairments found in children affected by FASD, which can be used to further explore the relationships between physiology and cognitive dysfunction.

Key words: FASD, cognitive dysfunction, alcohol, *in utero*, behavioral test, rat, behavior

Table of Contents

List of Tables & Figures

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Consequences of In Utero Exposure to Alcohol
With a Focus on Behavioral Outcomes

1. Introduction

In utero exposure to alcohol can result in fetal alcohol spectrum disorder (FASD), which encompasses a range of developmental disorders (CDC, 2019; Brust, 2010). Physical abnormalities paired with cognitive defects are typical of someone diagnosed with FASD (CDC, 2019). Abnormalities can include atypical facial features, low body weight, reduced memory capacities, learning disabilities, low IQ, hyperactive behavior, etc. (CDC, 2019). The prevalence of FASD has been estimated to impact 20-50 children per 1,000 births with an estimated economic burden of 6-10 billion dollars/year (CDC, 2019). There has been no safe amount of alcohol consumption indicated during pregnancy, nor a window of time in an active pregnancy where drinking alcohol is considered safe (CDC, 2019). Alcohol can be easily passed from mother to fetus as alcohol in the blood can be delivered to the fetus through the umbilical cord (CDC, 2019). Brain development occurs throughout the entirety of the gestational period which is why there is no safe window to consume alcohol during the process (CDC, 2019). Any disruption in brain development can cause behavioral/cognitive abnormalities which are indicated in our experiment.

FASD is a prevalent problem within the United States, considering between the years of 2015-2017 11.1% of pregnant women indicated they have consumed alcohol in the past 30 days; 3.9% of those individuals indicated binge-drinking tendencies (Denny, et al., 2019). The importance of raising awareness to the disorder can inform people of the dangers associated with consuming alcohol while pregnant. The general public may be aware that drinking during a pregnancy may be dangerous, but they may not know the

extent of the damage that can be caused (Anderson et al., 2014). There are inconsistencies in the data surrounding levels of drinking recommendations to pregnant women, and the safest route in the literature suggests fully abstaining from alcohol consumption throughout the extent of the gestational period (Anderson et al., 2014). Inconsistencies across the board from physicians, peers, scientists, etc. leads to confusion by pregnant mothers which calls for collection of clearly stated data (Coons et al., 2017). There is a particular lack of information about the possible harms associated with low level consumption of alcohol during pregnancy, but a lack of data does not suggest safety (Coons et al., 2017). This is why we are conducting research to target gaps in the data available in the literature to better explain the range of cognitive defects caused by FASD. The importance of making reliable data available is essential especially for medical professionals who communicate with pregnant mothers about the dangers of consuming alcohol during their pregnancy (Coons et al., 2017). The presentation of clear-cut facts about the harmful effects of alcohol consumption during gestational periods could aid in lowering the rates of FASD (Coons et al., 2017).

In utero exposure to alcohol results in impairment to nitric oxide synthase dependent dilation of cerebral arterioles and an increase in damage to the brain from ischemia (Cananzi & Mayhan, 2019). FASD individuals are at a greater risk for cognitive dysfunction, dementia, and seizures, possibly due to impaired cerebral vascular function (Brust, 2010; Cananzi & Mayhan, 2019). Determining whether *in utero* exposure to alcohol alters reactivity of cerebral arterioles (neurovascular coupling) resulting in behavioral abnormalities will help identify underlying mechanistic causes of FASD. There is currently no known cure for FASD (CDC, 2019), and no specific treatment

strategies for either vascular or behavioral symptoms (Murawksi et al., 2015). Early home and school intervention can aid in support and education (National Organization on Fetal Alcohol Syndrome) to help parents and caregivers with available services. A team of specialists can help an individual with FASD navigate speech problems, physical symptoms, cognitive issues, and psychological disturbances (Mayo Clinic, 2018). Additionally, there are medications available that may control symptoms associated with FASD but cannot cure the underlying disorder (Mayo Clinic, 2018). An early diagnosis of the disorder is necessary to help a child navigate symptoms and improve their quality of life (Mayo Clinic, 2018).

Rats have been a useful animal model to represent human defects for many years in science, and are the preferred rodent model for many cardiovascular and behavioral studies (van der Staay et al., 2009; Bader 2010), with social, spatial and working memory models of cognition particularly relevant (Berman & Hannigan 2000; Scholl et al., 2019). For these reasons, we chose male and female rats as our model organism for a method to investigate the cognitive deficits caused by *in utero* exposure to alcohol. Including both male and female rats is important because using only male rats in science was common in the past since the males had no interference of an estrous cycle (Beery & Zucker 2011; Will et al., 2017; Scholl, et al., 2019). However, male and female rats have behavioral, cognitive and social differences similarly to male and female humans (Berman & Hannigan, 2000; Becker et al., 2005; Scholl, et al., 2019). To address these issues, females are included, and the stages of estrous were recorded in the event of cycle effects on behavior (Scholl et al., 2019). We tracked the estrous cycle in the female rats throughout our experiment by vaginal lavage for future use in determining any potential

role in estrous cycle-related behaviors. These procedures ensure the most accurate reflection of human females, as the estrous cycle in rats correlates to that of the human female menstrual cycle (Becker et al., 2005; McLean et al., 2012; Scholl, et al., 2019).

2. Hypothesis and Predictions

Our central hypothesis is that *in utero* exposure to alcohol contributes to cognitive dysfunction, resulting in memory deficits and abnormal behaviors associated with FASD. We expect that rats exposed to alcohol *in utero* will exhibit deficits from cognitive impairment that is positively correlated with impaired vascular reactivity, as well as to deficits in short- and/or long-term memory. Our goal is to identify the underlying causes of physiological and behavioral effects of FASD to better understand the disease.

3. Method

3.1 Animals

Pregnant Sprague-Dawley rats were fed an alcohol diet (3% alcohol) or control diet throughout their gestational period. Pups were cross-fostered to account for differences in maternal care, and weaned at 3 weeks old. Following weaning, the female and male pups were pair-housed within same treatment / same sex groups on a reverse 12 h light 12 h dark cycle with free access to food and water, maintained at 22°C, and 60% relative humidity. Rats were utilized in the experiment first at 4-6 weeks old for the adolescent period testing, and again at 14-16 weeks for the adult phase of testing. All behavioral testing and animal handling was conducted between 11am and 7pm. Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Dakota.

3.2 Behavioral testing procedure

Behavioral testing was performed during the dark phase under dim red lighting (approximately 18 lx) in a semi-random order (Table 1), with 48-72 hours between each test. Male and female alcohol-exposed and control rats (Table 2) were tested for behavioral/cognitive deficits at 4-6 weeks old and 14-16 weeks old.

Table 1: Example of semi-random behavioral test design

Test 1	**Test 2**	**Test 3**
NOR	SI	T-Maze
NOR	SI	T-Maze
T-Maze	NOR	SI
T-Maze	NOR	SI
SI	T-Maze	NOR
SI	T-Maze	NOR
T-Maze	NOR	SI
T-Maze	NOR	SI
SI	T-Maze	NOR
SI	T-Maze	NOR

Table 2: Animal numbers for each behavioral test and treatment group

		Adolescent Alcohol	Adolescent No Alcohol	Adult Alcohol	Adult No Alcohol
NOR	Male	10	8	10	8
	Female	10	6	10	6
T-Maze	Male	9	10	9	10
	Female	12	6	12	6
SI	Male	10	7	10	7
	Female	11	6	11	6

The Spatial T-Maze, Novel Object Recognition, and Social Interaction tests were chosen as well-validated tests of working memory, spatial/long-term memory and social behavior. Tests were conducted on each rat a total of 2 times throughout the experiment. During the 14-16-week testing phase, the female rats were examined once daily via vaginal lavage to determine the stage of their estrous cycle.

3.2.1 Estrous Cycle Determination.

Vaginal lavages were conducted once daily throughout the adult testing phase. Vaginal secretion samples were collected with a glass pipet containing sterile 0.9% saline and examined under a microscope on a clean, glass slide to determine the stage of their estrous cycle (Scholl, et al., 2019). The determination was made by two independent researchers and a third researcher was called in to verify the cycle if a discrepancy was observed. Vaginal lavages were performed once daily and stages of estrous (proestrus, estrus, diestrus and metestrus) were evaluated to track the female estrous cycle. Cell types were determined (epithelial, cornified, and leucocytes) where estrus contained primarily 100% cornified, diestrus primarily leucocytes and proestrus primarily nucleated epithelial and metestrus a distribution of cell types (McClean et al., 2012). A similar procedure was performed on the males to make sure handling was universal. All handling and experiments were conducted in a manner to limit human exposure. Similar handling was conducted as closely as possible throughout the extent of the experimental period for both the male and female rats.

3.2.2 Novel Object Recognition. The Novel Object Recognition (NOR) Test is a working memory experiment which investigates whether a rat can tell the difference between a familiar and novel object. On the first day of testing, a rat is placed in a

rectangular tub without any objects for 30 minutes. This 30-minute period is the habituation stage for the rat to become accustomed to the apparatus. On the second day, the rat is placed in the tub with either two bones or two balls attached to opposite sides of the tub with Velcro for 10 minutes. The 10-minute period to explore those two objects is called the familization stage. After the ten minutes, the rat is placed back in its cage for a 5-minute delay while the researcher cleans the tub and introduces a novel object to the rat. If the rat was placed in a tub with 2 bones, the researcher will remove one of the bones and introduce a novel ball. The same process would happen in a tub with 2 balls but vice-versa. The rat gets placed in the tub with a novel object for a 10-minute period during this test stage. During both days of trials, the computer software detects, records, and scores time spent with each object, as well as total distance traveled (Ethovision XT v5.1; Noldus Technologies). Distance traveled in habituation is analyzed to determine that no locomotor deficits have been introduced, and the discrimination index (DI) is determined for both familiarization trials and test trials to determine ratio of time spent with objects. The rat should be more inclined to explore the novel object if the rat's working memory is functioning properly, exhibited by a higher DI. Rats are curious creatures which is why introducing a novel object extracts many details about the rat's cognitive functioning.

3.2.3 Spatial T-Maze. This test is utilized to investigate spatial/long-term memory with a 24 hour delay. The T-maze consists of an uppercase "T"-shaped Plexiglas apparatus. There are two stages of the T-maze test. On day one of testing, the rat is placed in the starting arm of the maze for a 30-minute trial. One of the cross arms at the top of the T-maze is blocked off on either the right or left side during the day one trial

(Sathyanesan, et al., 2018). On the second test day, both arms are open for the rat to explore while the 10-minute trial ensues (Sathyanesan, et al., 2018). The Ethovision software detects and scores number of entries to each arm, total distance traveled, and time spent in each arm of the maze (Sathyanesan, et al., 2018). Similar to the NOR test, the rat should be able to remember the familiar side and be more inclined to explore the novel arm of the maze as a method to measure the rat's spatial/long-term memory capacity. Rats with impairments in long-term memory will show equal time spent in both arms of the maze. Time spent in the neutral arm is also calculated and subtracted from the ratio.

3.2.4 Social Interaction. The Social Interaction (SI) Test analyzes social behavior and possible anxiety in rat models. The SI test involves a black, oval-shaped apparatus (54.5x80cm) divided virtually into zones (each measuring 54.5x26cm) in which the rat is placed with an empty, wire mesh cage (Scholl, et al., 2019). For the familiarization step of the process, the rat is placed in the apparatus with the empty cage to get acquainted to the novel space for 30 minutes. Rats are briefly removed and returned to their home cage, then the rat is placed in the black tub for a second time for a 5-minute interaction period. The second trial includes a stimulus rat (same sex/same age) as the target rat placed in the mesh cage to measure the social interaction or avoidance between the rats (Scholl, et al., 2019). Rats are social creatures, so the target rat should be apt to explore the stimulus rat in the mesh cage, and avoidance would indicate social deficits or anxiety.

3.2.5 Data analysis. For all comparison a Grubbs' test (Grubbs, 1969) for statistical outliers was performed, and 18 data points were removed. Distance traveled during habituation and familiarization trials, as well as discrimination indices in the NOR

were compared amongst groups with separate 3-way repeated measure ANOVA (sex x treatment x repeated factor of age) and followed by separate two-way ANOVAs to evaluate the main effects. Pairwise comparisons were done by Student-Newman-Keuls (SNK) post-hocs when appropriate. For contextual long-term memory, percent time spent in each arm of the T-Maze was compared against hypothetical chance level (33% for this maze) using separate t-tests within each group. Distance traveled and percent time spent in the novel arm of the T-Maze was compared in a three-way repeated measures (RM) ANOVA (sex x treatment x repeated factor of age) and comparisons were made in the SI test across the same groups. Ratios were determined for total distance traveled, and time spent in the interaction zone of the test with a novel target rat and with a novel object (empty mesh cage), as well as for number of entries to the interaction zone. Main effects were further examined by separate two-way ANOVAs, and pairwise comparisons were done by Student-Newman-Keuls (SNK) post-hocs when appropriate. All three-way analyses were conducted with SPSS Statistics 20 (IBM Corp., Armonk, New York), and all other analyses were done using SigmaPlot v13.0. and alpha level was set at 0.05 throughout.

4. Results

4.1 Estrus Cycle Determination. Cycle lengths were determined from vaginal lavage, and averaged 4 ± 0.24 days. Length did not differ between alcohol and no alcohol treatment groups, with means of 3.875 ± 0.183 and 4.250 ± 0.479 respectively ($p = 0.389$).

Figure 1

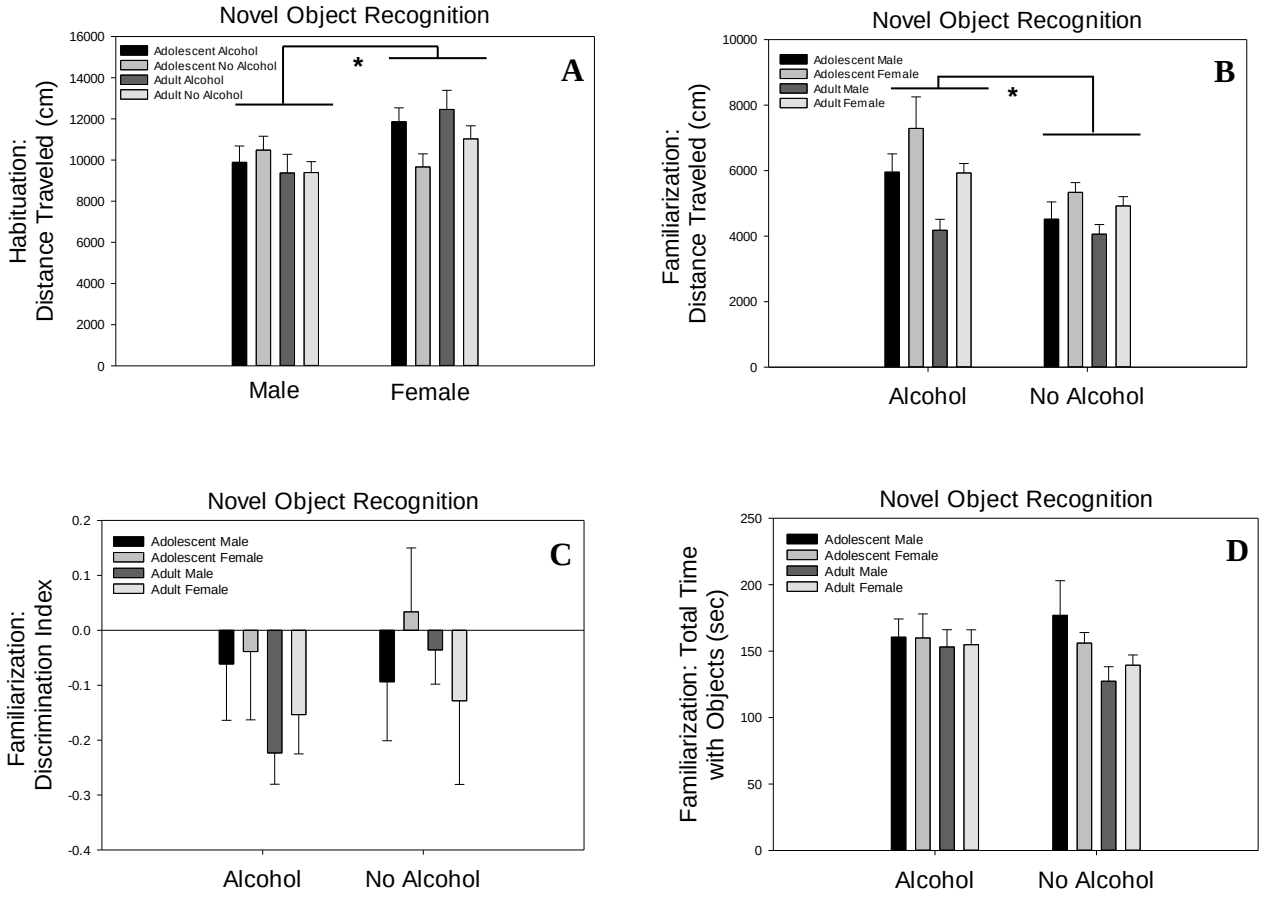

Figure 1: Novel Object Recognition with 5 Minute Delay; Habituation and Familiarization Trials

Female rats traveled significantly more than males in the habituation trial in the NOR test (A). Alcohol exposed rats traveled significantly more than control rats during the familiarization trial, as did females and adolescent rats (B); $p = 0.004$. No statistical differences were found between any group in discrimination index of identical novel objects (C), nor total time spent with either identical object (D).

4.2 Novel Object Recognition. To determine potential effects of *in utero* alcohol exposure on working memory, an initial three-way ANOVA compared effects between sex x age x alcohol treatment in the NOR test. During the habituation phase of testing, females traveled significantly more than males in overall distance (Figure 1A; $F_{(1, 26)} = 5.404$; $p = 0.028$).

Similarly, females traveled more than males during the familiarization trial of the NOR test (Figure 1B; $F_{(1, 29)} = 9.883$; $p = 0.004$), and alcohol-exposed rats also traveled more (Figure 1B; $F_{(1, 29)} = 9.876$; $p = 0.004$). Additionally, adolescent rats moved more than adult rats (Figure 1B; $F_{(1, 29)} = 5.120$; $p = 0.031$). Discrimination index (DI) is calculated to determine the ratio of time spent with one object in comparison to the other, and during the familiarization trial identical objects are used. No differences were found between alcohol groups (Figure 1C; $F_{(1, 28)} = 0.659$; $p = 0.424$), sex (Figure 1C; $F_{(1, 28)} = 0.382$; $p = 0.542$), or age (Figure 1C; $F_{(1, 28)} = 2.354$; $p = 0.136$), indicating no preference for object or side of testing chamber. Overall total time investigating objects did not differ between alcohol exposed rats and control (Figure 1D; $F_{(1, 28)} = 0.130$; $p = 0.721$), sex (Figure 1D; $F_{(1, 28)} = 0.073$; $p = 0.789$), or age; however, adolescent rats show a strong trend towards investigating objects in general (Figure 1D; $F_{(1, 28)} = 3.913$; $p = 0.058$).

Figure 2

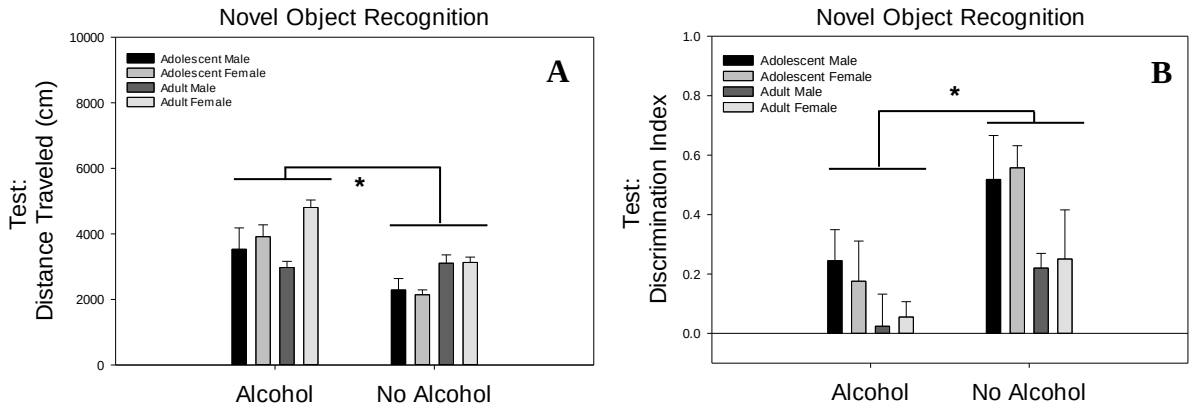

Figure 2: Novel Object Recognition with 5 Minute Delay; Test Trial

Alcohol exposed rats travel significantly more than control in the NOR test (A). Alcohol exposed rats have a lower discrimination index compared to controls, as do adult rats compared to adolescent (B).

Analysis of test day distance traveled revealed that the alcohol-exposed rats traveled significantly more during the test than control rats (Figure 2A; $F_{(1, 28)} = 10.258$; $p = 0.003$) regardless of age. This could suggest that alcohol exposure increases excitability and general locomotor activity in comparison to an age- and sex-matched control, and that this persists into adulthood. Adolescent rats exhibit a trend towards decreased distance (Figure 2A; $F_{(1, 28)} = 3.951$; $p = 0.057$), though not significant. Discrimination index was calculated to determine the ratio of time spent with the novel object in comparison to the familiar (a DI of 0 means no distinction was made and time spent with both objects is equal). Rats exposed to alcohol *in utero* have a significantly lower DI than control rats, regardless of age or sex (Figure 2B; $F_{(1, 29)} = 7.113$; $p = 0.012$), suggesting a deficit in working memory, and an inability to remember the familiar object, thus equally investigating both. Adolescent rats have a significantly higher DI than adult rats regardless of alcohol exposure or sex (Figure 2B; $F_{(1, 29)} = 4.761$; $p = 0.037$) indicative of their increased interest in novel objects in general.

Figure 3:

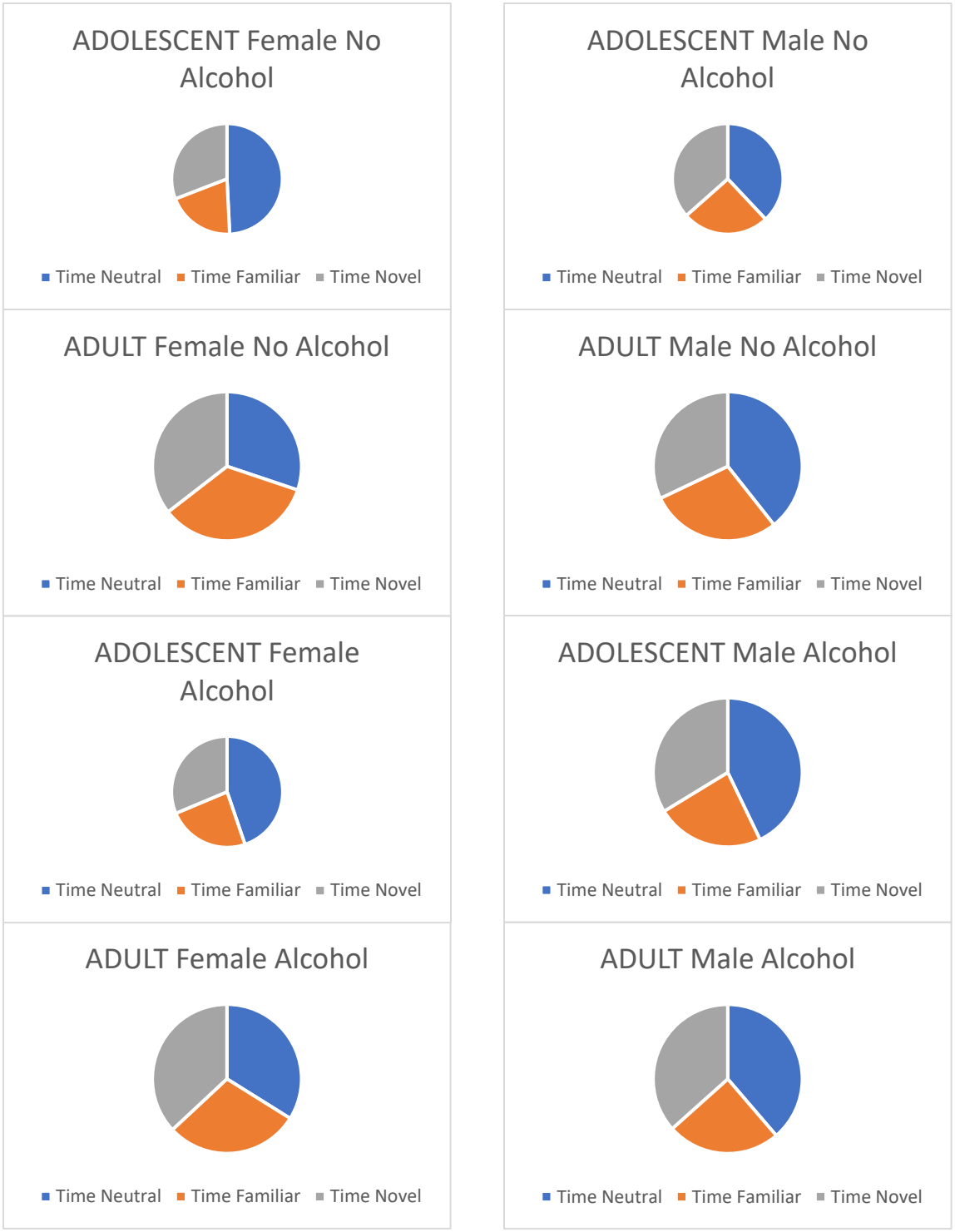

Figure 3: Visualization of % Time in T-Maze Arms
Percent time in each arm against chance (33%) was compared within all groups. No differences from chance were found within any group within any arm ($p > 0.05$).

Figure 4:

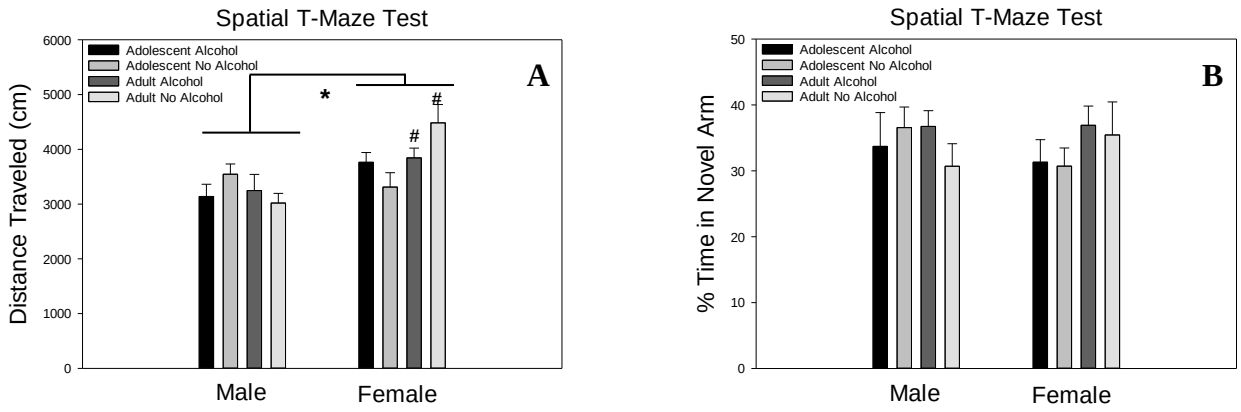

Figure 4: Spatial T-Maze with 24 Hour Delay

Female rats traveled significantly more compared to males in the maze, and adult female rats traveled more than adult males. No treatment differences were found (A). No differences between any group were found in percent time spent in novel arm within the total test time of the spatial T-Maze (B).

4.3 Spatial T-Maze. To determine effects of long term memory in a spatial context, the rats were tested in an alternating T-Maze with a 24-hour delay. Percent of time spent in novel arm against chance was compared with a t-test (Table 3) and no significant differences were found within any group (Figure 3; $p > 0.05$ for all tests).

Age	Female No Alcohol	Male No Alcohol	Female Alcohol	Male Alcohol
Adolescent	0.39	0.33	0.57	0.94
Adult	0.69	0.46	0.24	0.44

Table 3: T-test p-values for % time against chance

Distance traveled across groups (age x sex x alcohol treatment) was analyzed with a 3-way repeated measures ANOVA. Post hoc analysis revealed a significant two-way ANOVA interaction between age x sex (Figure 4A; $F_{(1,35)} = 4.233$; $p = 0.047$) with females driving that effect compared to males (SNK, $p < 0.001$). During the test phase, females traveled significantly more compared to males in the maze, and adult female rats traveled more than adult males (Figure 4A; $F_{(1,33)} = 5.556$; $p = 0.024$). No treatment differences were found within any group. Again, during the testing phase, no differences were found between any group in percent time spent in novel arm within the total test time of the spatial T-Maze. No significant interactions were found in any group with a 3-way repeated measures ANOVA (Figure 4B; $F_{(1, 33)} = 1.013$, $p = 0.423$), or within any other comparison ($p > 0.05$ for all analyses).

4.4 Social Interaction / Avoidance Test. To determine any alterations in social behavior in addition to testing done for cognitive behavior, interactions between an age/sex matched rat were tested in a three-way mixed design ANOVA (age x sex x alcohol) with age as the repeated measure. Data for all social interaction behavior is reported as a ratio

(target/no target) for all the tests. The Social Interaction test indicated no difference in distance ratio (distance traveled with target/distance traveled with no target), no main effects and no interactions were found (Figure 5A; $p > 0.05$) for all analyses. There was a significant interaction between age and sex in ratio of entries to the interaction zone (Figure 5B; $F_{(1,28)} = 6.314$, $p = 0.018$). Post hoc analysis revealed that the male rats have a higher ratio of entries than females (Figure 5B; SNK, $p < 0.001$) and that adult males are making more entries to the interaction zone than adolescent males (Figure 5B; SNK, $p = 0.027$). A significant interaction occurred between age x sex within the total time spent ratio (Figure 5C; $F_{(1,29)} = 4.315$, $p = 0.047$). Post hoc analysis revealed that in contrast to ratio of entries to the interaction zone, with the ratio of total time spent in the interaction zone, adult males spend less time with the target rat than adolescent males (Figure 5C; SNK, $p = 0.050$) or than adult females (Figure 5C; SNK, $p = 0.044$). Overall, there was no treatment effect, indicating that social behaviors, as measured by interaction/avoidance, are not altered by *in utero* exposure to alcohol when tested with same age/same sex novel rats.

Figure 5:

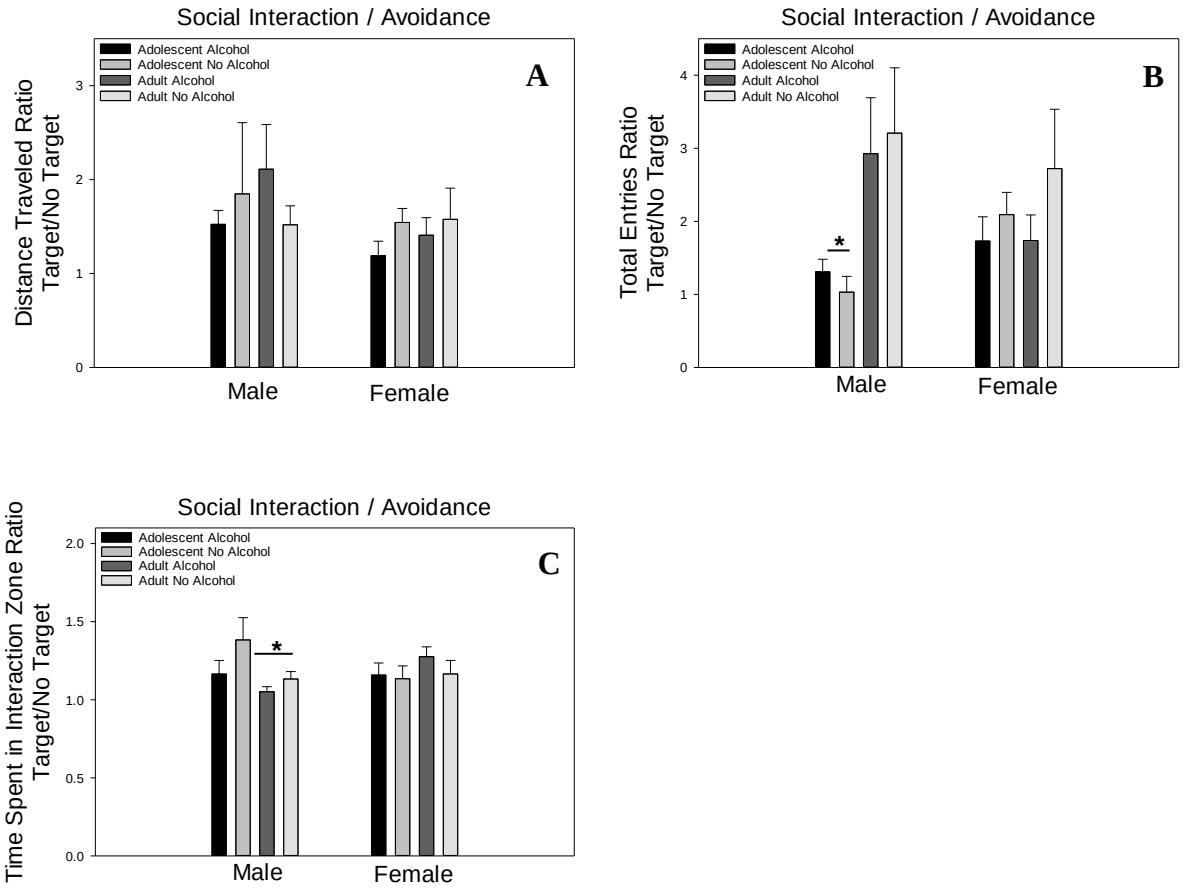

Figure 5: Social Interaction / Avoidance Behavior

For all analyses, ratio of measure with a target rat in the interaction zone of the chamber over measure with no target rat (novel object, empty cage) is reported. For total distance traveled, no significant interactions or main effects were found in any group (A). For ratio of total entries, a significant interaction between age x sex was observed, with adult male rats making more entries to the interaction zone (B). The ratio of time spent with the target rat revealed that adult male rats spent significantly less time with the target rat compared to other groups (C).

5. Discussion and Conclusion

Fetal alcohol spectrum disorder is a multifaceted disease that is a prevalent problem in the United States (CDC, 2019). Issues arising from *in utero* alcohol exposure can range from minor developmental anomalies and cognitive deficits to fully diagnosable Fetal Alcohol Syndrome, which constitutes a very specific pattern of physical, behavioral, and cognitive defects (Hoyme et al., 2005). Despite being first identified in the 1970s (Jones & Smith, 1975), FASD continues to be a problem. Exploring the relationship between physical and behavioral effects of *in utero* alcohol exposure and helping to determine underlying mechanisms involved in FASD could lead to a better understanding of the disease.

Animal models of FASD are used to explore learning and behavioral deficits, and spatial memory is typically most reported (Berman & Hannigan, 2000). The current study sought to test working memory, spatial/long-term memory as well as social behaviors to approach the wide range of reported deficits in humans. The current results suggest specific behavioral changes following *in utero* alcohol exposure, depending on task. Rats exposed to alcohol *in utero* exhibit deficits in novel object recognition, suggesting cognitive impairment in working memory tasks (Figure 2B). These results indicate that alcohol-exposed rats have difficulty distinguishing between an object they have already explored, compared to a new object. Rats are curious creatures, so they should be naturally more apt to explore a new object rather than a familiar object. A decline in working memory inhibits the rats from distinguishing a new object from a familiar object. Alcohol-exposed rats exhibit this deficit regardless of age and sex, which suggest that this impairment lasts across time points and into adulthood. Overall, adolescent rats have a

higher discrimination index compared to adult rats in the NOR Test (Figure 2B), regardless of treatment or sex, which is consistent with reports of natural novelty seeking in adolescence (Stansfield & Kirstein, 2005). This type of behavior can be explained because adolescent rats may be more curious which causes them to spend more time with the novel object and give themselves the opportunity to explore objects more in-depth. Age differences in DI could also be related to natural cognitive decline from adolescence throughout adulthood (Bizon et al., 2012; Mota et al., 2019).

Female rats exhibit greater distance traveled in both the Novel Object Recognition (Figure 2A) and T-Maze test (Figure 5A), similar to previous reports of increased locomotor behavior in female rats (Scholl et al., 2019). Female rats appear to exhibit less anxiety-like behavior in some measures, consistent with previous reports (Scholl et al., 2019; Mohammad et al., 2016); however, the increased locomotion behavior may play a role in primarily motor-based tests is often representative of anxiety-like behaviors in rats (Scholl et al., 2019). Furthermore, alcohol-exposed rats also displayed increased locomotion in both the NOR familiarization trial (Figure 1B) and the NOR test (Figure 2A) similar to previous reports of increased locomotor activity in rats affected by *in utero* exposure to alcohol (Hellemans et al., 2016). This effect could be an indicator that *in utero* exposure to alcohol results in heightened locomotor activity that could be interpreted as increased behavioral reactivity. These behaviors would align with the behavioral effects that we already know affect children with FASD. Individuals with FASD are more prone to anxiety disorders and depressive (Coffin et al., 2005) behavior than those in a control group (Hellemans et al., 2016).

No differences were found within any group in recognition between novel and familiar arms of the T-Maze suggesting that memory deficits induced with this model are specific to short-term working memory as opposed to long-term spatial memory (Figures 3-4). Rats appeared to spend equal amounts of time in all 3 available arms (Figure 3), and no significant differences were found in percent of time spent in the novel arm (Figure 4B). Behavioral impulsivity, attentional focus, and cognitive planning are all possible deficits reported with FASD (Kodituwakku & Kodituwakku, 2014), so long-term memory deficits may be more apparent in a test that requires training sessions. Current results indicate that short-term working memory is altered, but long-term spatial memory as tested by the T-Maze appears unaffected. The testing parameters could be at fault when trying to understand long-term spatial memory deficits. A 1-day delay may not be a long enough window of time to determine long-term memory deficits specifically resulting from *in utero* alcohol exposure, and a 3-day delay may be more appropriate when testing long-term spatial memory (Matthews & Simson, 1998). A proper window of time between the habituation period and the testing phase may be essential for testing certain cognitive deficits (Matthews & Simons, 1998). Long-term memory seems to decay more when given a 72-hour window compared to a 24-hour window in higher alcohol doses (Matthews & Simons, 1998). Considering long-term memory is for storing information over an extended period, increasing the period between habituation and testing, introducing a training session with no delay (Nagahara & Handa, 2006), or alternating the visible spatial cues may be necessary. Another option to consider is a possible overload on tests for a single rat. We tested each rat in 3 paradigms during adolescence and repeated the testing in adulthood which could have an impact on their

performance (Berman & Hannigan, 2000) even though there was a 48-hour washout period between each experiment. It is also possible that experience in a specific test during adolescence could have a carryover effect for the test in adulthood, especially in measures of long-term cognitive deficits.

Children with FASD are reported to have a range of social behavioral deficits, including aggression, self-regulation, and social skill deficits (Tsang et al., 2016). To address this issue along with cognitive dysfunction in our model, we assessed social interaction/avoidance behaviors with same-age same-sex target rats. Adult female rats spent more time with the target rat when compared to adult males (Figure 5C), no differences were seen in social behavior between the adolescent rats. An interesting result from the Social Interaction test was found wherein adult male rats make more frequent entries in the interaction zone (Figure 5B), but the adult males actually spent less total time with the target rat compared to the other groups (Figure 5C). This may suggest that the adult male has curiosities, but social anxiety may take over causing the rat to leave the interaction zone and choose not to interact with the stimulus rat. The male cannot simply be considered more active because there is no overall difference in the distance traveled ratio. Overall, there is no effect of alcohol exposure on social behaviors at least when the rats were tested with same age/same sex novel rats, suggesting that the 5-minute exposure to a target rat may not be a sensitive enough test to identify deficits in social skills in this particular model. In other studies, alcohol exposure *in utero* changed aspects of social behavior in rat models (Lugo Jr. et al., 2003), so we will look into adjusting our parameters.

Taken together: *In utero* alcohol exposure results in short-term working memory deficits that persist into adulthood. The current model highlights short-term working memory deficits but was unable to identify long-term/spatial cognitive dysfunction. To further explore the behavioral outcomes of *in utero* alcohol exposure, training sessions and separate cohorts of age groups will be utilized to better identify cognitive dysfunction in the model of FASD. Following refinement of behavioral measures for long-term/spatial memory, this model will serve to examine the components of cognitive, social, and physiological impairments resulting from *in utero* alcohol exposure.

Future Directions: To determine appropriate measures for long-term/spatial memory testing for adolescent and adult alcohol-exposed rats. Following that, to test if there is a functional relationship between impaired cognitive function and vascular reactivity in the FASD rat model.

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