An analysis of locomotion, anxiety, and recognition memory across development in a Fetal Alcohol Spectrum Disorder rat model

Undergraduate Research Thesis

Presented in partial fulfillment of the requirements for graduation with Research Distinction in Psychology in the undergraduate colleges of The Ohio State University

by

Nicole MacIlvane

The Ohio State University
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Project Advisor: Professor Derick Lindquist, Department of Psychology
Abstract

Alcohol consumption during pregnancy may result in Fetal Alcohol Spectrum Disorders (FASD), which are often characterized by impairments in cognition. Our laboratory employs a FASD rat model in which pups are exposed to ethanol across postnatal days (PD) 4 through 9, a period roughly comparable to the human third trimester. We previously demonstrated impaired hippocampus-dependent learning and memory in adult (PD 70) FASD rats, as assessed by fear-elicited freezing behavior. It is unclear, however, whether the deficit is due specifically to hippocampal dysfunction, aberrant input from upstream brain regions (e.g., the perirhinal cortex, PR), or altered behavioral patterns (i.e., anxiety, hyperactivity). To dissociate these variables between FASD and control rats over development we measured locomotion (open field, OF) in adolescent (PD 35) subjects, and locomotion, anxiety (passive avoidance, PA) and memory (novel object recognition, NOR) in adult rats. In the OF, quantification of gridline crossings revealed adolescent 5E rats were more active than SI rats within the periphery, and all adolescent rats became more active across the three sessions. Adolescent rats also demonstrated increased anxiety from session 1 to 3. Adult animals showed no significant differences in the OF across sessions or treatment groups, but females were more active than males. For PA, adult rats were placed in a white, brightly lit chamber separated from a black, unlit chamber and allowed to explore for 5 min. Decreased time in the brightly lit arena would indicate increased anxiety, albeit no treatment group differences were observed. Finally, in the PR-dependent NOR task subjects were exposed to two identical objects then tested on their ability to discriminate between the familiar and a novel object 20 min or 4 h later, representing short-term memory (STM) and long-term memory (LTM), respectively. Results suggest FASD rats are not impaired in the STM or LTM variants of the NOR task. Together, results imply that 5E rats are more hyperactive than SI rats as adolescents, but as adults 5E rats perform similarly to controls for locomotion, anxiety and object recognition.
Introduction

Fetal Alcohol Spectrum Disorder

Fetal Alcohol Spectrum Disorders (FASD) result from maternal alcohol consumption during pregnancy, affecting 2-7 children in every 1,000 births (May et al., 2009). Offspring may suffer from physical irregularities (Streissguth et al., 1980; Barr & Streissguth, 2001) including growth disturbances, facial dysmorphology, spine and organ dysfunction (Abel & Sokol, 1987; Smitherman, 1994). FASD may cause behavioral and cognitive impairments as well (Streissguth et al., 1980; Barr & Streissguth, 2001), and is known to be among the leading causes for preventable mental retardation in the US (Abel & Sokol, 1987; Stratton, Howe & Battaglia, 1996). Cognitive deficits include: lower IQ (Mattson et al., 1996), difficulty perceiving social cues (Streissguth et al., 1991), deficits in attention, information processing, and executive functioning (Nanson & Hiscock, 1990; Lee, Mattson & Riley, 2004; Kodituwakku et al., 1995; Roebuck, Mattson & Riley, 1999); and can be seen with or without physical markers of FASD (Clarren et al., 1992). Current research aims to understand the underlying pathophysiology of FASD in order to alleviate cognitive symptoms.

Neuroimaging studies have shown an overall decrease in brain volume and white matter due to fetal alcohol exposure (Archibald et al., 2001), especially in areas involved in cognition such as the cerebellum, amygdala, and prefrontal cortex (Bookstien et al., 2001; Spadoni et al., 2007; Coles et al., 2002). These changes in neuroanatomy along with neurophysiological alterations (Fagerlund et al., 2006; Alfonso-Loeches & Guerri, 2011) across the neocortex and cerebellum contribute to impairments in cognition. In
particular, learning and memory deficits are attributed to ethanol-induced neurodegeneration of the hippocampus (HC) and surrounding areas (Bhatara et al., 2002; West, Hamre & Cassell, 1986) such as the medial prefrontal cortex (Mantha, Kleiber & Singh, 2013).

Our lab administers ethanol to postnatal rats in order to model FASD. Pups are exposed to ethanol across postnatal days (PD) 4-9, a time-period roughly comparable to the third trimester in human development, characterized by a “growth spurt” in which brain areas grow and solidify connections (Bayer et al., 1993; Gil-Mohapel et al., 2010). By exposing rats to ethanol during the postnatal period, FASD models have displayed neurodegeneration of the cerebellum and HC, as well as cognitive dysfunctions similar to FASD humans (Onley et al., 2002; Mantha et al., 2012; Johnson & Goodlett, 2002). For example, animals exposed to ethanol during the postnatal period exhibit deficits in spatial, associative, and contextual learning as well as executive functioning tasks (Goodlett & Peterson, 1995; Hunt, Jacobson & Torok 2009; Hamilton et al., 2011; Blanchard, Riley & Hannigan, 1987; McClelland & Goddard, 1996).

Recent studies in our laboratory indicate FASD model rats are impaired in hippocampal-dependent associative learning. These deficits were apparent when adult animals were tested in a variant of one-trial contextual fear conditioning (Goodfellow & Lindquist, 2014) and a cognitively challenging form of cued fear conditioning, called trace fear conditioning (Dupont et al., 2014). It is unclear, however, whether the impairment is due specifically to HC dysfunction, aberrant input from brain regions upstream of the HC (e.g., the perirhinal cortex, PR), or altered behavioral patterns (i.e., anxiety, hyperactivity). To dissociate these variables between FASD and control rats and
describe behavior over development, we measured locomotion in adolescent animals, and locomotion, anxiety and memory in adult animals.

**Changes in Behavior across Development**

In humans, FASD is known to correlate with Attention Deficit Hyperactivity Disorder (ADHD), impulsivity, and hyperactive behaviors (Bhatara, Loudenberg & Ellis, 2006). These increases in hyperactivity are evident in rodent models as well (Driscoll, Streissguth & Riley, 1990; Hellemans et al., 2010; Wigal & Amsel, 1990; Clarren et al., 1992), although there are a few exceptions (Carneiro et al., 2005; Dursun, Jakubowska-Dogru & Uzbay, 2006). The mechanism underlying this hyperactivity is unknown, but studies suggest FASD rats are deficient in response inhibition (Driscoll et al., 1990) – i.e., the do not have the same degree of top down control. Some studies find that the intensity of this hyperactivity varies as a function of age as well as ethanol dose: in a review, Bond (1981) came to the conclusion that if animals are prenatally exposed to more than 6 g/kg/day of ethanol and tested prior to PD 70 they will exhibit hyperactivity, due to the delayed maturation of inhibitory networks (Berman & Hannigan, 2000).

Consequently, we set out to investigate if similar hyperactive behavior is seen in FASD rats when tested during adolescence (PD 32) and young adulthood (PD 65). The open field task (OF) allows for the observation of an animal’s locomotor and exploration behaviors within an open arena containing a grid lined floor. It relies on rat’s tendency to explore novel environments.

Many behavioral tasks have revealed that rats exposed to ethanol at different perinatal time points (i. e. 1st, 2nd, and/or 3rd trimester-equivalent exposure) show
increased anxiety behaviors (e.g. decreased time spent in the center, increased fecal boli) as adolescents and young adults (Hellemans et al., 2010; Mantha et al., 2013; Dursun et al., 2006; Ogilvie & Rivier, 1997; Osborn et al., 1998; Weinberg, Taylor & Granoulakis, 1996). It is suggested that this increase in anxiety is due to hyperresponsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to stressful stimuli (Mantha et al., 2013; Kim, Osborn & Weinberg, 1996).

Behavior in the OF task was used to explore possible alterations in anxiety across development due to postnatal ethanol exposure. Rats have a tendency to remain close to the walls of the open field while exploring (thigmotaxis), and decreased time spent in the center of the field relative to the periphery is reflective of increased anxiety (Prut & Belzung, 2003). We employed a modified version of the passive avoidance (PA) task to further examine possible changes in anxiety in adult FASD rats. In this paradigm, adult animals were exposed to an arena consisting of a brightly lit, white chamber and an unlit, dark chamber. The PA task is based on the rodent’s innate aversion to brightly lit, open areas and their spontaneous exploratory behavior when exposed to mild stressors (Bourin & Hascoët, 2003) such as the brightly lit chamber. A decrease in both time spent in the white chamber and latency to exit the white chamber are considered to be manifestations of increased anxiety in this task. These behavioral responses to the PA were measured to characterize anxiety in adult animals exposed to ethanol during the postnatal period.
Novel Object Recognition

Our lab has previously shown the deleterious effects of ethanol on associative learning and memory (Dupont et al., 2014; Goodfellow & Lindquist, 2014). We explored other possible learning and memory deficits through the Novel Object Recognition (NOR) paradigm, a simpler non-associative learning task that is highly dependent on PR function (Barker et al., 2007; Bussey, Muir & Aggleton, 1999). NOR relies on the tendency of rats to spontaneously explore novel objects in their environment (Ennaceur & Delacour, 1988).

Previous research has emphasized the importance of PR activity in different recognition tasks through lesions and drug interactions. The PR receives input from the ventral visual stream and other secondary sensory areas. Information flows downstream from the PR to the HC through polysynaptic connections, for example the entorhinal perforant pathway, as well as monosynaptic direct connections (Brown & Xiang, 1998; Buckley, 2005). The PR also relays information to other areas involved in recognition memory such as the prefrontal cortex, amygdala and thalamus (Brown & Xiang, 1998; Aggleton & Mishkin, 1983).

PR lesioned rats show significant object recognition impairments at both short and long delays, but no deficits in spatial memory (Bussey, Muir & Aggleton, 1999; Baxter & Murray, 2001; Barker & Warburton, 2011; Aggleton et al., 2010). Contrarily, lesions of the HC, but not PR, lead to deficits in spatial tasks but minimal dysfunction in recognition memory (Mumby, 2001; Kinnavane et al., 2014; Broadbent, Squire & Clark, 2004; Aggleton & Brown, 2005). This dissociation in memory impairment between
structures indicates the HC is necessary for spatial memories, but not object recognition, and vise versa for the PR.

Studying the PR allowed us to further understand the cognitive phenotype of FASD animals using a new learning and memory paradigm. Both the PR and HC contribute to recognition performance (Squire, Wixted & Clark, 2007) and their interaction is critical for object-in-place and temporal order memories (Barker & Warburton, 2011). However, modifications can be made to ensure object recognition tasks are solely dependent on PR function (Barker & Warburton, 2011). Additionally, data suggests acute or chronic alcohol exposure in adult and adolescent animals leads to structural and functional changes in the PR (Crews et al., 2000) and deficits in NOR (Pascual et al., 2007). These results led us to hypothesize that ethanol exposure at the critical third trimester equivalent period will result in greater alterations in PR function, and therefore worse performance in NOR. By focusing specifically on the PR, we can consider the possibility that areas upstream of the hippocampus are under performing in FASD rats. This damage to afferent inputs could contribute to observed disruption of HC function (Dupont et al., 2014).

Studies that investigated the effects of perinatal ethanol exposure on recognition memory in humans and non-human primates have shown deficits in visual recognition tasks compared to controls (Uecker & Nadel, 1996; Clarren et al., 1992). There have only been a few studies to look at PR cortex function in a FASD rat model (Jablonski et al., 2013; Summers et al., 2008). Jablonski et al. (2013) established that rats postnatally exposed to ethanol were not impaired in NOR when trained then tested 5 min later (i.e., a 5 min training-test interval [TTI]).
In the current study, adult FASD and control rats were first exposed to two identical objects and then tested on their ability to recognize the now familiar object versus a novel object. The NOR task was modified by using identical familiar objects and surrounding the open field arena with a black tarp to eliminate extramaze cues, ensuring that the task was solely dependent on the PR (Barker & Warburton, 2011).

We were also interested in looking at the short-term memory (STM) capacity of FASD rats in the NOR task. We employed a 20 min TTI to tax the rats abilities and investigate a possible short-term NOR impairment that was not evident in Jablonski et. al. (2013). We also tested long-term memories (LTM) in the NOR using a 4 h TTI. Research has established that NOR tasks that utilize a TTI less than 3 h are dependent on the cholinergic system, specifically muscarinic receptors within the PR (Warburton et al., 2003), while a TTI 3 h or longer is dependent on glutamate transmission and N-methyl-D-aspartate (NMDA) receptors (Nilsson et al., 2007; Lima et al., 2005; Winters & Bussey, 2005). Such data suggests that the aberrant behavior seen in the NOR task after a STM or LTM TTI may serve as a possible representation of deficits in the underlying neurotransmitter systems. If NMDA receptors in the PR are dysfunctional, similar to that seen in the HC (Goodfellow & Lindquist, 2014; Dupont et al., 2014), then LTM, in particular, is predicted to be impaired in the NOR task.

This thesis was designed to characterize the behavioral and cognitive phenotype of FASD animals over development, specifically focusing on locomotion, anxiety, learning and memory. Research in different FASD animal models have indicated changes in both locomotion and anxiety behaviors at different points in development (i.e., in juveniles and adults) (Riley et al., 1993; Mattson & Riley, 1998; Dursun et al.,
2006). These alterations in behavior can have an affect on the many learning and memory paradigms used to examine dysfunctions in FASD model rats, including tasks used previously in our lab (e.g., freezing behavior in fear conditioning paradigms). Characterization of the behavioral and cognitive phenotypes of our FASD model animals permits us to control for possible confounds that may influence our future behavioral results.

Methods & Materials

General Information

Breeding & Housing

Long Evans breeder rats were maintained with *ad libitum* food and water on a 12 h light/dark cycle (lights on at 0600 h). One male and female were pair-housed for one week; the female was checked daily for parturition beginning 3 weeks later. After birth, pups were housed with their mother, then weaned at PD 21 and same-sex housed with 2-3 littermates through PD 60. Following PD 60, animals were singly housed throughout testing and training. All procedures were conducted in accordance with The Ohio State University’s Institutional Animal Care and Use Committee (IACUC), and all necessary measures were taken to minimize pain and discomfort.

Neonatal Treatment

A total of 61 rats were used in this study. On PD 3, pups were paw marked for identification and separated into 3 different treatment groups: ethanol exposed (5E),
sham-intubated controls (SI) and unintubated controls (UC). Throughout PD 4-9, 5E pups experienced two intragastric intubations per day, first with an ethanol-milk solution of 5 g/kg/day, containing 11.33% ethanol by volume. The second intubation occurred 2 h later and consisted of a milk-only solution to maintain body weight. SI animals underwent the same intubation procedure as 5E animals, but did not receive the ethanol-milk or milk solution. UC rats were removed from their home cage along with the SI and 5E rats, but did not experience any intubations.

**BAC analysis**

Immediately before the final intubation on PD 4, blood was collected from 5E and SI rats via a tail clip procedure. Approximately 20 μL of blood was collected from both groups of animals into capillary tubes. Blood samples from 5E animals were then transferred into micropipette tubes and centrifuged for 10 min. Plasma samples were collected and analyzed for blood alcohol concentration (BAC) using an Analox GL5 Analyzer (Analox Instruments, Lenenberg, MA). The Analox calculated BAC by measuring the rate of oxygen consumption resulting from ethanol oxidation in samples versus a known alcohol standard.

**Experimental Design**

For each task employed, the focus was on behavioral differences between treatment groups, analyzed as a function of session, age and sex. 5E, SI and UC rats were initially introduced to the OF during adolescence. As adults, all treatment groups
were exposed to PA, introduced to a second OF, then tested in NOR (Table 1). All tasks were video recorded with a Panasonic HC-V720 high definition video camera and analyzed offline by an observer blind to treatment groups. All arenas, transfer boxes and objects used in behavioral testing were wiped with 70% ethanol between every session to clean surfaces and eliminate possible odor cues.

**Behavioral Tasks**

**Adolescent Open Field Task**

Behavioral testing began with the OF in adolescent rats (~PD 33-35). The task consisted of 3 sessions lasting 5 min, ~24 h apart. The context was an open field (22 x 24 x 12.125 in) with white plastic walls and black gridlines spaced 6.25 in apart. Animals were transferred from their home cages, in which they were group-housed with 1 or 2 littermates, via black transfer boxes. They were placed in the center of the open field facing the northern wall. Rats were allowed to explore the open field for 5 min, then returned to their home cages via the same transfer box. White noise (65 dB) was played throughout the task to diminish background sounds. Total, peripheral, and center gridlines crossed by the rats were recorded as measures of locomotion. Time spent in the center and periphery of the open field as well as number of fecal boli were recorded as measures of anxiety. Finally, we used a novel measure to examine the rate at which
animals were moving, calculated as gridlines crossed per second in the periphery and center of the open field.

**Passive Avoidance Anxiety Task**

Anxiety testing occurred on ~PD 65, when rats were exposed to a passive avoidance chamber. One side of the chamber had a dark, black-painted arena (12.125 x 12.125 x 12.125 in) which was separated from an illuminated, white-painted arena (12.125 x 12.125 x 12.125 in). A 40 W bulb was placed 12 in above the white arena, and at the level of the animal the light intensity reached 250 lux. A hole 5 in wide allowed animals to move between chambers. The rats were transferred to the testing room in their home cages, then placed in the middle of the white-painted chamber, facing away from the black chamber. They were allowed to explore both chambers for a total of 5 min. The latency for animals to initially leave the white arena and enter the black arena and time spent in the white arena were determined as measures of anxiety—i.e., decreased latency to enter the black arena and decreased time in the white arena corresponding to increased anxiety.

**Adult Open Field Task**

The OF occurred again on ~PD 66-68 for all rats. The task consisted of three 5 min sessions, ~ 24 h apart, and was employed to allow habituation to the NOR context along with examination of adult rat locomotor behavior. Adult animals were retrieved from their home cages via a black transfer box and placed in the center of the open field
facing the northern wall. A second OF was used to assess locomotion in adult rats, and consisted of an arena (24 x 24 x 16 in) with black walls and gridlines spaced 6.5 in apart. Unlike adolescent OF, the entire arena was encompassed 360° by a black curtain 70.25 in high to eliminate any extramaze cues outside of the open field that would engage the HC. Number of total, peripheral and center gridlines crossed in the center vs periphery were counted as measures of locomotion. Time spent in the center and periphery and number of fecal boli in the arena at the completion of each session were counted as measures of anxiety. The rate at which animals were moving was also determined based on gridlines crossed per second in the periphery and center of the open field.

**Novel Object Recognition**

*Training.* At ~PD 70 animals underwent NOR training. Rats were transported from their home cages to the test room via a black transport box. The NOR context consisted of the same arena used for adult OF, again surrounded by the black curtain, which allowed us to assess habituation to the NOR context for locomotion. During the training session, two identical objects were placed in the open field, 12 in apart from one another and 5 in away from the closest wall. The animal was placed in the center of the field, facing away from the objects and allowed to explore for 5 min, then returned to its home cage.

*Objects.* Two distinct objects were used throughout training and testing. The first was a transparent green water bottle made of non-porous plastic. The bottle was 8.5 x
3.5 in and was filled halfway with water during NOR to prevent animals from pushing the bottle over. The second object was a brown glass bottle, which was 8.25 x 2.5 in. There were 3 replicas of each object, 2 randomly assigned per animal to be used during the training phase and one that was used during the testing phase. The objects were duct taped to the floor of the open field to ensure they could not be moved by the animal.

**Testing.** For the NOR test, animals were returned to the training context either 20 min or 4 h later in order to test STM and LTM, respectively. One of the familiar objects (F1) in the context was replaced with a novel object (N), and the other familiar object (F2) was replaced with an identical “familiar” object (F3). The usage of a new identical object (F3) during testing eliminated possible confounds that could arise from using the same familiar object encountered during training (F1 or F2), for example odor cues or slight alterations between “identical” object’s appearances. The animal was again placed in the center of the field, facing away from the objects and allowed to explore for 5 min. The placement of F3 and N were counterbalanced between groups.

**Analysis.** To determine exploration rates during training, videos were rated for exploration of F1 & F2. Total time spent exploring each object was rated offline by a blind experimenter by stopwatch. Exploration of an object was defined as the rat having its nose ≤ 2 cm from the object, sitting on the object was not considered active exploration (Ennaceur & Delacour, 1988; Summers et al., 2008; Barker et al., 2007). The same analysis was done for test videos to determine exploration of N and F3. After 5 min, time spent exploring each object was rounded to the nearest second and recorded.
Statistical Analyses

A total of 61 rats were used for this study; in cases where more than 1 male or female per treatment group per litter were used within a behavioral task, data were averaged into a single data point. Data was analyzed using single-factor, multi-factor and repeated measures ANOVAs. A significant post-hoc (Tukeys HSD Test) test indicates $p < 0.05$.

Results

Neonatal Treatment

BACs

All blood plasma samples acquired from 5E rats via tail clips on PD 4 underwent analysis by a Analox GL5 Analyzer. The mean peak BAC in 5E rats was $395.1 \pm 13.3$ mg/dL. Due to equipment issues, BACs were not be analyzed for 11 rats, but previous studies from our lab suggest, once analyzed, we will find similar BAC levels for the remaining 5E rats.

Body Weights

Body weights for all male and female rats ($N=61$) were recorded across PD 4-9, 10, 15, 21, 30, 45 and 60 (Table 2). Body weights were analyzed during the period ethanol was administered (PD 4-9) and across development (PD 10-60). For postnatal exposure, a 3 (treatment) x 6 (PD) repeated measures ANOVA demonstrated significant effects for treatment group, $F(2, 58) = 3.30, p < 0.05$, PD, $F(5, 290) = 2120.38, p <$
0.001, and a significant treatment x PD interaction, $F(10, 290) = 24.72$, $p < 0.001$. One-way (treatment) ANOVAs for each PD revealed significant differences in body weight, with 5E rats weighing less than both SI and UC rats, for PD 7, $F(2, 58) = 7.80$, $p < 0.00$, PD 8, $F(2, 58) = 6.82$, $p < 0.00$, and PD 9, $F(2, 58) = 7.80$, $p < 0.001$. The same 3 x 6 repeated measures ANOVA administered for PD 10-60 weights showed a significant main effect for PD only, $F(5, 250) = 1037.78$, $p < 0.001$, indicating all animals demonstrated an increase in weight over time.

<table>
<thead>
<tr>
<th></th>
<th>PD 4</th>
<th>PD 5</th>
<th>PD 6</th>
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<th>PD 45</th>
<th>PD 60</th>
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<tbody>
<tr>
<td>UC (n=20)</td>
<td>9.15 ± 0.13</td>
<td>10.49 ± 0.19</td>
<td>12.16 ± 0.23</td>
<td>15.43 ± 0.23</td>
<td>17.13 ± 0.24</td>
<td>18.99 ± 0.27</td>
<td>29.66 ± 0.50</td>
<td>46.00 ± 0.82</td>
<td>92.58 ± 1.64</td>
<td>187.09 ± 1.64</td>
<td>274.32 ± 11.92</td>
<td></td>
</tr>
<tr>
<td>SI (n=20)</td>
<td>8.85 ± 0.34</td>
<td>10.34 ± 0.40</td>
<td>11.82 ± 0.50</td>
<td>15.08 ± 0.53</td>
<td>16.72 ± 0.59</td>
<td>18.72 ± 1.06</td>
<td>29.73 ± 1.79</td>
<td>45.7 ± 1.79</td>
<td>91.01 ± 2.56</td>
<td>179.95 ± 7.78</td>
<td>259.80 ± 13.12</td>
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<tr>
<td>5E (n=21)</td>
<td>9.28 ± 0.20</td>
<td>10.04 ± 0.25</td>
<td>11.17 ± 0.28</td>
<td>12.18 ± 0.34</td>
<td>13.51 ± 0.36</td>
<td>14.95 ± 0.38</td>
<td>16.63 ± 0.70</td>
<td>28.17 ± 1.17</td>
<td>44.84 ± 2.03</td>
<td>90.40 ± 6.97</td>
<td>183.89 ± 12.41</td>
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* Denotes a significant treatment group effect, $p < 0.001$.

Table 2. Mean ($\pm$ SE) body weight across PD 4 - 60 by neonatal treatment group. Across PD 7-9 5E rats weighed significantly less than SI and UC rats.

Adolescents

Open Field

As discussed above, when there was more than 1 sex per treatment group per litter, data points were collapsed. Consequently, ten animals (UC n= 2 males, 2 females; SI n= 2 males, 2 females; 5E n= 2 females) were collapsed into 5 data points. Thus, as adolescents, UC (n=19), SI (n=18) and 5E (n=19) rats were exposed to the OF task (N=56). Dependent variables analyzed in this task included fecal boli, total, peripheral and central gridlines crossed, time spent in the center and periphery, and gridlines crossed per unit of time (1 sec). Total gridlines were initially analyzed using a 3 (treatment) x 3 (session) x 2 (sex) repeated measures ANOVA. Results showed
significant main effects for treatment, $F(2, 50) = 3.39, p < 0.05$, and session, $F(2, 100) = 3.37, p < 0.05$. No significant effects for sex or interactions between treatment, sex and session were found, therefore OF data was collapsed across sex. Consequently, adolescent OF behavior was analyzed using 3 (treatment) x 3 (session) repeated measures ANOVAs.

*Fecal Boli.* As a measure of anxiety, the number of fecal boli that remained in the arena after each session was quantified (Fig. 1A). The 3 (treatment) x 3 (session) repeated measures ANOVA revealed no differences in boli number across sessions or treatments, as well as no interaction. This result implies that the level of stress from OF exposure was not sufficient to provoke treatment group differences in defecation.

*Total Gridlines.* Total gridlines crossed in the OF (Fig. 1B) is an index of overall locomotor activity. The 3 (treatment) x 3 (session) repeated measures ANOVA revealed a significant main effect for session $F(2, 106) = 3.90, p < 0.05$, with a significant increase between sessions 1 and 3, collapsed across treatment groups. The main effect for treatment ($p = 0.07$) showed a trend toward, but did not reach, statistical significance. The interaction of treatment x session was not significant.

*Peripheral Measures.* The number of peripheral gridlines crossed (Fig. 2A) is a metric of thigmotaxic behavior. The 3 (treatment) x 3 (session) repeated measures ANOVA indicated significant main effects for treatment, $F(2, 53) = 3.51, p < 0.05$, with an increase in locomotion for 5E rats compared to SI rats, and session, $F(2, 106) = 3.32, p < 0.05$, with all animals demonstrating more movement in session 3 than 1. However, no significant interaction for treatment x session was found. Results of the repeated measures ANOVA for time in the periphery only showed a significant main
effect for session, $F(2, 106) = 8.87, p < 0.001$, with all animals spending less time within the periphery between sessions 1, 2 and 3 (Fig. 2B). To better understand the rate at which animals were moving while in the periphery the number of gridline crossings per unit of time (1 second) was calculated and analyzed (Fig. 2C). The repeated measures ANOVA results demonstrated a main effect for treatment only, $F(2, 53) = 3.47, p < 0.05$, with 5E rats exhibiting an increase in activity relative to SI rats across sessions.

\textit{Center Measures.} Analyzing gridlines crossed within the center of the open field (Fig. 3A) allowed us to determine locomotor activity within a mildly anxiety-provoking environment. Results from the 3 (treatment) x 3 (session) repeated measures ANOVA did not reveal any statistically significant for main effects or interactions. Time spent in the center of the arena (Fig. 3B) was analyzed independently of time spent in the periphery to better understand possible changes in anxiety, with decreased time in the center indicative of increased anxiety. Comparable to results of time in the periphery, the repeated measures ANOVA indicated a significant session effect, $F(2, 106) = 8.80, p < 0.001$, due to animals spending less time in the center of the arena between sessions 1 and 2, and 1 and 3. Treatment group and the interaction of session x treatment did not reach significance. Analyzing the rate (gridlines/seconds) at which animals moved allowed for a better indication of motion during the possibly anxiety-provoking time within the center (Fig. 3C). The repeated measures ANOVA only showed a significant difference by session $F(2, 106) = 16.31, p < 0.001$. Post hoc analyses revealed all treatment groups were increasingly active within the center across sessions 1, 2 and 3.

Taken together, these results imply that all animals regardless of treatment became more active across sessions, especially in the periphery. Importantly,
adolescent 5E rats displayed hyperactivity compared to SI rats. When examining anxiety behavior, all treatment groups spent less time in the center across sessions.

**Adults**

*Passive Avoidance*

To control for same sex per litter per treatment group, ten rats (UC \(n=2\) males, 2 females; SI \(n=2\) males, 2 females; 5E \(n=2\) females) were collapsed into 5 data points. Additionally, six animals (UC \(n=1\) male, 1 female; SI \(n=1\) male, 1 female; 5E \(n=1\) male; 1 female) were not included in analyses because they had completed behavioral testing prior to the addition of PA to this study. The PA task allowed for a measure of anxiety in UC \((n=17)\), SI \((n=16)\) and 5E \((n=17)\) adult rats \((N=50)\) by quantifying their motivation to explore a naturally aversive environment. Increased time spent in the white, brightly lit arena and a longer latency to enter the black arena indicate decreased anxiety. A 3 (treatment) x 2 (area) x 2 (sex) ANOVA for time spent in the white area (Fig. 4A) revealed no significant differences for sex, treatment or their interaction. A 3 (treatment) x 2 (sex) ANOVA for latency to enter the black arena (Fig. 4B) indicated the sex x treatment interaction approached significance \((p = 0.09)\), suggesting a slightly increased latency for male 5E rats. These results imply that adult rats do not differ in anxiety behavior regardless of neonatal treatment or sex.

*Open Field*

To control for same sex per litter per treatment group, ten animals (UC \(n=2\) males, 2 females; SI \(n=2\) males, 2 females; 5E \(n=2\) females) were collapsed into 5
data points. Thus, UC \((n=19)\), SI \((n=18)\) and 5E \((n=19)\) rats were introduced to a novel OF arena as adults \((N=56)\) to habituate to the NOR task context and to investigate possible changes in locomotion and anxiety behavior at a later point in development. A 3 (treatment) x 3 (session) x 2 (sex) repeated measures ANOVA was initially analyzed to determine if males and females performed differently within treatment groups for total gridlines crossed. Results revealed no main effects for treatment or session, but a significant effect for sex, \(F(1, 50) = 12.07, p < 0.001\), due to females showing significantly increased activity relative to males. No interactions between treatment, session, or sex reached significance. To more concisely illustrate these results, all subsequent data is collapsed across sessions 1, 2 and 3 for each rat and a 3 (treatment) x 2 (sex) ANOVA was used to analyze all open field dependent variables.

*Fecal Boli.* The 3 (treatment) x 2 (sex) ANOVA indicated a significant sex effect, \(F(1, 50) = 4.2, p < 0.05\). This result demonstrates that males defecated more, and therefore were more anxious, than females (Fig. 5A). The effect of treatment group and the interaction of treatment x sex did not reach significance.

*Total Gridlines.* The 3 (treatment) x 2 (sex) ANOVA showed a significant sex effect, \(F(1, 50) = 12.07, p < 0.001\), suggesting that female rats were more active than male rats (Fig. 5B). No main effect for treatment group or treatment x sex interaction was seen.

*Peripheral Measures.* For peripheral gridlines crossed (Fig. 6A), the 3 (treatment) x 2 (sex) ANOVA revealed a significant effect for sex, \(F(1, 50) = 9.91, p < 0.001\), demonstrating that females were more active in the periphery than males. Effects for treatment or the sex x treatment interaction were not significant. When investigating
time spent in the periphery (Fig. 6B), the 3 x 2 ANOVA showed no significant main effects or interactions for treatment x sex, indicating no differences in anxiety for males and females across treatment groups. The 3 x 2 ANOVA for rate of movement (gridlines/second) within the periphery (Fig. 6C) again revealed a significant sex effect, $F(1, 50) = 10.83, p < 0.001$, with females exhibiting increased rate of movement within the periphery compared to males. Treatment and the interaction of treatment x sex did not reach significance.

**Center Measures.** A significant sex effect, $F(1, 50) = 7.86, p < 0.001$, for center gridlines (Fig. 7A) was detected by the 3 (treatment) x 2 (sex) ANOVA due to females showing more locomotion than males within the center of the arena. No significant effects were seen across treatment groups or in the treatment x sex interaction. The 3 x 2 ANOVA only revealed a significant sex effect for time spent in the center (Fig. 7B), $F(1, 50) = 5.02, p < 0.05$. This result demonstrates that females spent significantly more time within the center of the open field, indicative of decreased anxiety. The 3 x 2 ANOVA did not show any significant main effects or interactions for rate of gridlines crossed per second within the center (Fig. 7C).

Overall, these results indicate there are no differences in locomotor behavior across treatment groups or sessions when considering OF analyses. However, sex seems to exert a significant effect, as females displayed increased locomotion in the center, periphery and overall. Results of time in the center and periphery suggest stable anxiety levels across treatment groups and sessions, but females demonstrate increased anxiety behaviors compared to males.
Novel Object Recognition

Previously, behavioral data was collapsed for animals of the same sex, litter, and treatment group. Since animals were separated into either STM or LTM variants in the NOR task, animals previously collapsed could be analyzed separately. Also, sex was not analyzed as a factor due to smaller group sizes within STM and LTM tests.

To determine object exploration during each training and test session, time spent exploring each object was recorded per animal ($N=60$), then Exploration Ratios (ER) were calculated by dividing the time spent exploring an object of interest by the total time the rat spent exploring both objects during that specific session. The ERs were analyzed using one-way (treatment) ANOVAs for each object presented during training, then again for testing.

STM NOR Training and Test. UC ($n=11$), SI ($n=10$) and 5E ($n=11$) animals ($N=32$) were run in the NOR task using a 20 min TTI to selectively tax STM processes. The one-way ANOVA showed similar ER rates for UC, SI and 5E animals for both objects presented during the training session (Fig. 8A). These results demonstrate that all neonatal treatment groups showed similar exploration of the familiar objects. For the test session, the ANOVA revealed no significant treatment group differences for ER of the novel or familiar object (Fig. 8B). These results imply neonatal treatment had no effect on the ability of rats to recognize a familiar vs novel object at STM eliciting intervals.

LTM NOR Training and Test. The remaining UC ($n=8$), SI ($n=10$) and 5E ($n=10$) animals ($N=28$) were run in another version of the NOR task which used a 4 h TTI to focus on LTM processes. Similar to STM results, the one-way ANOVA revealed no
significant differences between treatment groups in the ER rates of either object during training (Fig. 9A). Results for the LTM test session were also similar to the STM test, with all treatment groups exploring the novel object more than the familiar object (Fig. 9B). These results demonstrate neonatal treatment had no significant effect on initial exploration of either object, or LTM object recognition.

Taken together, the data illustrates that our FASD model rats were not impaired in either initial object exploration or novel object recognition utilizing both short and long delays. These results suggest neonatal ethanol exposure does not affect PR-dependent NOR performance in adulthood.

**Discussion**

Across three behavioral tasks, the behavioral phenotype of 5E rats in regard to locomotion, anxiety and object recognition was explored throughout development. Results of adolescent OF revealed all rats show increased locomotion (Fig. 1B, 2B) and anxiety (Fig. 3B) across sessions, regardless of neonatal treatment. Additionally, 5E rats were significantly more active in the periphery than SI animals (Fig. 2A, 2C), indicating hyperactive behavior. Adult OF results do not show any treatment group or session differences, but female rats demonstrated increased locomotion relative to males (Fig. 5B, 6A, 6C). Females also exhibited decreased anxiety, spending more time in the center (Fig. 7B) and excreting fewer boli (Fig. 5A). Adult animals were also run in the PA task which is another measure of anxiety behavior. No differences were seen across sex or treatment groups (Fig. 4), although 5E males did show a trend toward decreased
latency to exit the anxiety-provoking white arena. Finally, adult rats were tested in a NOR task determining the efficacy of PR-dependent STM and LTM. Results revealed all animals are comparably successful in discriminating a novel from a familiar object (Fig. 8, 9).

**Fetal Alcohol Spectrum Disorder**

In-utero ethanol exposure in humans may cause a variety of physical, behavioral and cognitive impairments (Barr & Streissguth, 2001), and increased risk of developing additional disruptive behavioral disorders (e.g., ADHD, conduct disorder, and oppositional defiant disorder) (Bhatara et al., 2006), or mental retardation (Abel & Sokol, 1987). Although these dysfunctions can be measured through experimental tasks, it can be difficult to diagnosis and predict FASD without confirmation of maternal alcohol consumption during pregnancy (Smitherman, 1994). Furthermore, many developmental deficits are affected by timing, duration, and dosage of ethanol exposure (Berman & Hannigan, 2000) which poses a problem when trying to compare specific symptoms across FASD individuals (Driscoll et al., 1990). Since human and rat neurodevelopmental timelines are comparable, rat models of FASD are used to increase experimental control of ethanol exposure. Therefore, FASD rat models can provide more reliable data concerning behavioral and neurobiological abnormalities.

Binge-like exposure to ethanol in postnatal rodent life or during the third trimester in humans often leads to a variety of deficits in spatial, contextual, and associative learning tasks, as well as executive functioning (Goodlett & Peterson, 1995; Hunt et al., 2009; Hamilton et al., 2011). These disturbances in function are due to ethanol-induced
neurodegeneration in areas such as the HC, PFC and cerebellum, which are highly involved in learning and memory (Mantha et al., 2013). Although many aberrations are seen in early in life (Nagahara & Handa, 1997; Riley, 1990), ramifications of ethanol exposure on the developing brain areas can persist through adulthood (Johnson & Goodlett, 2002).

Previous research from the Lindquist lab has shown evidence of dysfunctional HC-dependent learning in adult rats following PD 4-9 ethanol exposure. Specifically, 5E animals exhibit deficits in consolidating a context memory that is predictive of an aversive footshock stimulus when training and testing were separated by a long (24 h), but not short (2 h) interval (Goodfellow & Lindquist, 2014). Dupont et al. (2014) also found ethanol-induced impairments in trace fear conditioning, in which subjects must associate a tone with an aversive footshock when both stimuli are separated in time. Both of these Pavlovian association tasks are heavily influenced by learning-dependent plasticity in the HC. These results, along with many others throughout the literature (West et. al., 1986; Mantha et al., 2013), reveal evidence for HC dysfunction due to neonatal ethanol exposure.

It is important to consider that alterations in locomotion and anxiety due to neonatal ethanol exposure can influence an animals behavior, producing seemingly significant aberrations when none exist. For example, impairments in attention (Kodituwakku et al., 1995; Roebuck et al., 1999), sensory-motor systems, and habituation to stimuli (Driscoll et al., 1990) are often seen in FASD model rats and could affect performance in learning and memory tasks. By examining anxiety and locomotion in adult ethanol-exposed and control animals, this thesis refutes several possible
confounds that could negatively influence HC-dependent learning and memory paradigms employed in our lab.

**Locomotion in Adolescents & Adults**

The OF was first employed in adolescent rats to investigate locomotion behavior at an early stage of development. When examining open field behaviors across age, Bronstein (1972) found that adolescent rats display increased locomotor activity across sessions. Similarly, adolescent rats in the current study showed increased overall locomotion between sessions 1 and 3 as measured by total gridlines crossed (Fig. 1B). Additionally, adolescent animals exhibited an increase in peripheral gridlines crossed between sessions 1 and 3 (Fig. 2A), although this may be due to rats spending more time in the periphery during session 3 (Fig. 2B).

Possible locomotor alterations due to postnatal ethanol exposure were investigated as well. Hyperactivity is one of the most common behavioral effects of FASD in both animal and human studies (Barron & Riley, 1988). Children suffering from FASD show a high risk for an ADHD diagnosis (Bhatara et al., 2006), but these behaviors decrease over time (Driscoll et al., 1990). Correspondingly, research concerning activity in adolescent rats show ethanol-induced hyperactivity compared to controls when administered during the postnatal period (Wigal & Amsel, 1990; Riley et al., 1993), although the age of adolescents in these studies were slightly younger than those tested in this study, ranging from PD 16-20. Many studies in which ethanol was administered throughout gestation also indicate increased activity at slightly older adolescent time points (PD 28-35) (Bond & Giusto, 1977; Marche, Danel & Bordet,
2011; Driscoll et al., 1990). Results from the current study support hyperactive behavior in FASD model adolescent rats– 5E rats showed an increase in peripheral gridlines crossed compared to SI rats (Fig. 2A). This effect is further supported by the fact that time spent in the periphery did not affect the increase in peripheral gridline crossings, as 5E animals also demonstrated an increased rate of motion (gridlines/second) in the periphery compared to SI animals (Fig. 2C). Although the increased activity of 5E rats did not reach significance when compared to UC rats, SI rats underwent both intubation and tail clip procedures and therefore serve as a better control group than the UC rats (Lindquist et al., 2013).

Ethanol-induced hyperactivity in adolescents is well supported in the literature, however a few studies have found different results. Tran et al. (2000) and Mantha et al. (2013) demonstrated increased activity in rats exposed to prenatal ethanol, but rats exposed to ethanol in the postnatal period behaved similar to controls. It is possible that this lack of hyperactivity in animals exposed to postnatal ethanol is affected by the stress of ethanol injections or intubation procedures, which prenatally exposed animals do not experience. Additionally, this effect could be influenced by differences in length and levels of ethanol exposure during gestation (i.e., administered to dam and taken up differentially by pups) versus postnatally (i.e., direct exposure). It should also be considered that Mantha et al. (2013) showed hyperactivity in rats exposed to ethanol during the postnatal period by analyzing home cage activity at certain peaks throughout the night.

Results of hyperactivity in adult FASD rats are somewhat controversial (Dursun et al., 2006). Some studies have observed increased activity in adult animals exposed
to ethanol at different time points (Bond & Giusto, 1976; Bond & Giusto, 1977; Osborn et al., 1998; Tran et al., 2000), although these studies (besides Tran et al., 2000) administered ethanol during gestation only. The majority of studies suggest adults are not hyperactive and display locomotor behavior similar to controls when exposed to ethanol during the pre- and postnatal period (Abel and Berman, 1994; Randall & Hannigan, 1999; Westergren et al., 1996; Chen & Smith, 1979; Dursun et al., 2006). In accordance with the majority of previous research, all adult rats in this study displayed similar levels of locomotion regardless of treatment group (Fig. 5B, 6A, 6C, 7A, 7C).

Results of this study indicate that ethanol exposure during the postnatal period alone does not cause locomotor alterations in adult rats, although adolescent rats demonstrate increased activity. This dissociation in adolescent and adult activity is seen throughout the FASD literature (Bond & Giusto, 1977; Abel, 1982). Neonatal ethanol exposure is thought to delay the development of inhibitory systems, causing a deficit in response inhibition prior to puberty (Barron & Riley, 1988). As animals abilities to inhibit exploration or habituate to a novel environment are compromised (Driscoll et al., 1990), the impairment in top-down control leads to hyperactivity.

Although neonatal treatment did not affect overall activity in adult rats, sex proved to play a significant role. Females showed increased locomotor activity in multiple measures, including total (Fig. 5B), peripheral (Fig. 6A) and center gridlines crossed (Fig. 7A), and rate of movement in the periphery (Fig. 6C). The discordant effects of sex in adolescent versus adult animals indicates the onset of puberty leads to dissociations in behavior between males and females. Results from both the adolescent and adult OF suggest that initially, pre-pubescent male and female rats behave similarly.
but once puberty is reached females show elevated locomotion compared to males. This increase in female locomotion is seen in OF tasks independent of FASD (File, 2001; Gould, Dao & Kovacsics, 2009; Brocardo et al., 2012), and is due to the absence of testosterone during neonatal life and the presence of ovarian secretions in adulthood (Blizard, Lippman & Chen, 1975).

Alterations in OF activity are often seen across the lifespan. Usually, locomotion increases from adolescence (~PD 30) to early adulthood (~PD 60), reaches a peak within mid-life (~PD 90), then declines throughout old age (~PD 120) (Gould et al., 2009; Chen & Smith, 1979). In order to directly contrast overall locomotor behavior in the adolescent and adult OFs (specifically total gridlines crossed), a 3 (treatment) x 2 (sex) x 2 (age) repeated measures ANOVA was analyzed, producing a significant age effect, \(F(1, 50) = 4.83, p < 0.05\), with adults more active than adolescents. Further, results also revealed a treatment x sex, \(F(2, 50) = 3.82, p < 0.05\), and age x sex, \(F(1, 50) = 13.72, p < 0.001\), interactions. Post hoc testing indicated 5E females exhibited increased activity compared to all other sexes and treatment groups independent of age. Also, adult female rats crossed significantly more gridlines than adult male rats and adolescent male and female rats. These alterations in activity across age may arise from size differences in adolescent and adult animals. Since adult rats are larger than adolescent rats, it is possible that they are able to cross more gridlines with less effort, leading to a higher number of gridlines crossed. It is also important to note that animals were exposed to two different open field contexts during adolescence and adulthood, and variation in the color, size and location of the open field could add confounding factors to locomotion results.
Anxiety Behavior in Adolescents & Adults

When considering behavioral changes that could affect aversive associative learning tasks, aberrant anxiety would be an extremely undesirable confound, as it could cause alterations in fear responding (e.g., freezing behavior). To determine if 5E animals showed any changes in anxiety as adolescents or adults, OF sessions were analyzed for time spent in the center (Fig. 3B, 7B), and number of fecal boli excreted (Fig. 1A, 5A). The number of fecal boli is an index of autonomic nervous system activation (Gould et al., 2009) and emotionality of animals (Bond & Giusto, 1977) triggered by individual testing in an arena larger than the rat’s usual habitat (Prut & Belzung, 2003). Time in the center reflects anxiety levels since rats typically prefer to remain close to the walls of the open field (Prut & Belzung, 2003; Gould et al., 2009).

Previous research demonstrates postnatal ethanol exposure leads to increased levels of anxiety when tested in the OF as adolescents (Wigal & Amsel, 1990; Kleiber, Wright & Singh, 2011; Mantha et al., 2013), possibly due to ethanol-induced dysfunction of the HPA axis (Dursun et al., 2006). When using different behavioral tasks such as the light/dark box, FASD adolescent rats do not show alterations in anxiety (Mantha et al., 2013), although these results could be attributed to different levels of stress or motivation to explore during tasks rather than ethanol-induced changes in behavior. Results from adolescent rats do not support our prediction that 5E rats would display altered anxiety in the OF, as no treatment group differences were found for any anxiety related measures (Fig. 1A, 3B).
Regardless of neonatal treatment, adolescents spent less time in the center between sessions 1 and 2, and 1 and 3 (Fig. 3B). We cannot attribute this decreased time in the center to an overall decrease in exploration of the open field, as animals demonstrated an increase in number of gridlines crossed across sessions (Fig. 3A). Interestingly, rats also moved at an increased rate in the center across sessions (Fig. 3C), which may reflect the rats attempt to quickly flee the anxiety-provoking area. Taken together, these results indicate that all adolescents may be experiencing increased anxiety across subsequent exposures to the OF.

Although we predicted to see alterations in adult OF anxiety measures due to postnatal ethanol exposure, results showed that neonatal treatment had no effect on anxiety (Fig. 5A, 7B). Similarly, studies by Chen & Smith (1979) and Brocardo et al. (2012) revealed similar anxiety levels in animals exposed to ethanol during the pre- and postnatal periods versus controls, as measured by time spent in the center of the open field and number of fecal boli. These results indicate that ethanol exposure during gestation and/or the postnatal period does not lead to alterations in anxiety for adult rats when tested in the OF test. It is worth noting that anxious behaviors in the OF task have been suggested to related more to emotional behavior, instead of being specific to anxiety (Royce, 1977).

To further investigate anxiety, adult rats were exposed to the PA. Results of this task revealed that postnatal ethanol exposure does not cause significant changes in anxiety levels based on time spent in the aversive (white) versus non-aversive (black) arena (Fig. 4A), or latency to exit the white arena (Fig. 4B). Other studies which utilized passive avoidance or the light/dark box procedure to examine effects of postnatal
ethanol exposure on anxiety also showed no differences between FASD and control animals (Mantha et al., 2013; Cronise et al., 2001). Dursun et al. (2006) and Osborn et al. (1998) argue that FASD adult rats are typically hyper-responsive to stressors, which may imply that neither the OF nor PA were anxiety-provoking enough to cause differential behaviors between 5E and control rats. Studies which used more stressful tasks, such as the elevated plus maze, established increased anxiety in adult FASD rats (Dursun et al., 2006) which was not seen in the OF task (Brocardo et al., 2012). Together, results suggest that anxiety is only elevated in FASD rats when exposed to highly stressful tasks, and those used in this study were insufficient to cause significant differences between 5E and control rats.

Similar to locomotion results, sex was a significant factor for anxiety behavior in the adult, but not adolescent, rats. Females exhibited increased time in the center compared to males (Fig. 7B), which indicates decreased anxiety. Although many other studies have established females spend more time in the center relative to males (Cao et al., 2010), it is important to consider that this increased time in the center may be due to increased activity levels in females (File, 2001). Fecal boli data shows that males defecate significantly more within the OF (Fig. 5A), which also reflects increased anxiety compared to females. Overall, anxiety measures in OF indicate that females are less anxious than males, but this sex effect does not influence behavior until after puberty. Previous studies suggest this difference in anxiety is due to hormones released during the estrus cycle, as females usually show decreased anxiety during estrus compared to diestrus (Burke & Broadhurst, 1966; Walden, 1968; Birke & Archer, 1975).
Object Recognition

Lastly, we utilized the NOR task to assess PR function in 5E, SI and UC rats. The PR receives sensory information from unimodal and polymodal sensory cortical areas (Buckley, 2005), especially visual areas (Brown & Xiang, 1997). It associates different visual views and various non-visual attributes of objects (Murray & Richmond, 2001) forming conjunctive representations (Bartko et al., 2007). When experiencing objects, the PR makes judgements concerning familiarity as well and will show a specific decrease in neuronal activity when animals are exposed to a familiar versus novel object (Brown & Banks, 2014; Warburton et al. 2013).

The PR and HC are closely integrated (Aggleton & Brown, 2005). Main efferents of the PR are to the entorhinal cortex, which then innervates the HC by way of the perforant pathway (Buckley, 2005). Both areas interact during object-in-place and temporal order memories (Barker & Warburton, 2011), however, a dissociation exists between the PR and HC. Individually, the PR is responsible for object recognition and familiarity while the HC is instrumental for spatial and contextual information (Aggleton & Brown, 2005, Wan et al., 1999; Brown & Xiang, 1998). Although NOR is known to be highly dependent on PR function (Barker et al., 2007; Bussey et al., 1999), the task used in this study was modified to ensure it relied solely on the PR. A curtain surrounded the OF arena, eliminating any context or extramaze cues that could engage the HC. Also, identical familiar objects were used during training to ensure the task remained PR dependent, whereas using two different familiar objects would involve the HC (Barker & Warburton, 2011).
During NOR training, animals did not display preferential exploration of either familiar object based on location or object type. Additionally, animals did not show any differences in exploration of the familiar objects across treatment groups (Fig. 8A, 9A). These results indicate that postnatal ethanol exposure does not affect the animal’s visual acuity or motivation to explore, and any differences seen in testing would be due to learning and memory deficits. For testing, we used 2 different training to test intervals (TTI), representing STM (20 min) and LTM (4 h).

For STM, rats were tested for novel object recognition 20 min after training. Antagonizing the PR cholinergic system disrupts subjects’ ability to respond to a novel object with delays less than 3 h (Brown et al., 2012). Specifically, muscarinic receptor antagonists lead to deficits at short (i.e., 20 min) intervals (Warburton et al., 2003). Previous work indicates that postnatal ethanol exposure does not produce impairments in this task with a 5 min TTI (Jablonski et al., 2013). However, exposing rats to prenatal ethanol leads to NOR deficits later in life when the TTI was lengthened to 15 min (Summers et al., 2008). Therefore, we employed a 20 min TTI so possible impairments in object recognition could be discerned in 5E rats. No significant differences between treatment groups were seen— all animals preferentially explored the novel object (Fig. 8B). These results demonstrate that the PR cholinergic system does not suffer detrimental effects due to postnatal ethanol exposure, at least as measured by this one task.

Another study tested the effect of prenatal ethanol on STM NOR and did find deficits at a 15 min delay (Summers et al., 2008), albeit there were multiple disparities in their experimental design compared to our own. For example, they exposed pregnant
mice to ethanol on gestational day 8 only, producing high BACs of ~500 mg/dL. Also, offspring were exposed to the NOR task at old age (~PD 120), whereas our rats were introduced to the task earlier in life (~PD 70).

In the LTM variant of the NOR test, animals were returned to the test 4 h after object exploration. The NOR literature suggests that object recognition at long intervals is supported by synaptic weakening processes, or long term depression (LTD) (Brown & Xiang, 1998). These processes are dependent on NMDARs and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), excitatory glutamate receptors which play a key role in the synaptic strengthening and weakening necessary for associative learning (Fanselow & Polous, 2005). Blocking or antagonizing either of these receptors leads to deficits in the NOR task when TTIs longer than 3 h are used (Brown et al., 2012; Winters & Bussey, 2005; Brown & Banks, 2014). Contrary to our hypothesis, then, 5E animals again showed no object recognition deficits suggesting glutamate transmission and plasticity in the PR is within optimal range (Fig. 9B). It is important to note, however, that the task was more difficult for all animals relative to the 20 min TTI, indicated by less exploration of the novel object. In the future, the addition of a shorter interval dependent on LTM (e.g., 2 h) could uncover treatment group differences.

Conclusions

Through multiple experimental paradigms, this thesis examined the behavioral and cognitive phenotype of FASD model rats over development. Results indicate adolescent 5E rats are hyperactive compared to SI rats, but all treatment groups have
similar levels of anxiety. Increases in locomotor behavior are often seen in adolescent FASD rat models (Wigal & Amsel, 1990; Riley et al., 1993), but this hyperactivity is usually rectified by adulthood (Bond & Giusto, 1977; Abel, 1982; Abel, 1986). Consistent with these findings, our results showed adult 5E rats display activity levels similar to SI and UC rats. Similar to adolescents, adult rats did not demonstrate alterations in anxiety due to neonatal treatment. We also investigated the effect of sex on locomotion and anxiety behavior, and found that adult female rats were more active and less anxious than adult male rats. Male and female adolescent rats, however, displayed comparable levels of locomotion and anxiety, implying such disparities in behavior arise after puberty due to hormonal changes. Additionally, results from the NOR task reveal no treatment group differences, suggesting postnatal ethanol exposure does not induce novel object recognition impairments at short and long TTIs.

Overall, these results indicate that postnatal ethanol exposure does not lead to alterations in locomotion or anxiety in adult rats that could adversely affect tasks dependent on the animals behavior. Behavioral tasks occurring during adolescence, on the other hand, should control for ethanol-induced hyperactivity. Additionally, previous work in our lab demonstrates 5E rats are deficient in aversive associative learning (Dupont et al., 2014; Goodfellow & Lindquist, 2014), which is dependent on a distributed neural circuit including both the PR and HC. Since 5E rats were not impaired in a PR-dependent NOR task, it is proposed that afferent input from the PR to the HC is not disrupted in our rat model of FASD. Therefore, learning and memory deficits previously seen in our lab are most likely due to the deleterious effects of postnatal ethanol on HC function, and not aberrant information processing within the PR. By examining
locomotion and anxiety across development, this thesis provided a behavioral and cognitive characterization of our FASD model rats, enhancing the face validity of our previous results (Dupont et al., 2014; Goodfellow & Lindquist, 2014).
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References


LOCOMOTION, ANXIETY AND OBJECT RECOGNITION IN FASD


Figure 1. Adolescent OF: Number of fecal boli and total gridlines crossed per session. (A) The number of excreted fecal boli did not significantly differ between treatment groups or training session. (B) Total gridlines crossed was significantly affected by session, but not neonatal treatment. All treatment groups demonstrated significantly increased locomotion in session 3 compared to session 1, denoted by *.
Figure 2. Adolescent OF: Peripheral Measures (A) Peripheral gridlines crossed was significantly affected by treatment group and session. All treatment groups exhibited increased locomotion in session 3 compared to session 1, denoted by *. Also, 5E rats were significantly more active than SI rats, denoted by #. (B) Time spent in the periphery of the arena significantly decreased for all treatment groups across sessions 1, 2 and 3. * Denotes this significant session effect. (C) Treatment significantly affected the rate of peripheral gridlines/second: 5E rats moved at a significantly higher rate than SI rats, denoted by #.
Figure 3. Adolescent OF: Center Measures (A) Number of gridlines crossed in the center did not significantly differ between treatment groups or sessions. (B) Time spent in the center significantly decreased across sessions 1 vs 2, and 1 vs 3, denoted by *. (C) The rate (gridlines/second) at which rats moved in the center showed a significant session effect. All treatment groups became more active from session 1 to session 3, denoted by *.
Figure 4. Adult Passive Avoidance. (A) Time spent in the white area (out of 300 s) did not significantly differ between sex or treatment groups. (B) Latency to exit the white area was not affected by treatment group or sex.
Figure 5. Adult OF: mean fecal boli and total gridlines crossed (A) Results indicate males excreted a significantly higher number of fecal boli. $ Denotes significance effect for sex collapsed across treatment group. (B) Females showed significantly increased locomotor activity compared to males, independent of treatment group. $ Denotes the significant sex effect.
Figure 6. Adult OF: Peripheral Measures (A) Females showed significantly more activity in the periphery compared to males, independent of treatment group. $ Denotes significant sex effect. (B) Amount of time spent in the periphery did not significantly differ between sexes or treatment groups (C) The rate of peripheral gridlines crossed was significantly affected by sex, but not treatment group. Females traversed significantly more gridlines per second than males within the periphery, denoted by $. 
Figure 7. Adult OF: Center Measures (A) Amount of gridlines crossed was significantly affected by sex, but not treatment group. Females crossed significantly more gridlines in the center of the open field than males, denoted by $. (B) Males spent significantly less time in the center than females. $ Denotes a significant sex effect independent of treatment group. (C) The rate of motion in the center did not significantly differ between treatment groups or sexes.
Figure 8. Adult STM NOR. Dashed line at 0.5 indicates chance levels of exploration. (A) Identical objects presented during training showed similar exploration ratios across treatment groups, at about chance levels. (B) Treatment groups did not show significant differences in exploration of the novel object.
Figure 9. Adult LTM NOR. Dashed line at 0.5 indicates chance levels of exploration. (A) Identical objects presented during training showed similar exploration ratios across treatment groups, at about chance levels. (B) Treatment groups did not show significant differences in exploration of the novel object.