Moderate Prenatal Ethanol Exposure, Sex, and Housing Modify Spatial Navigation Behavior and Hippocampal Cell Firing in Adult Rodents

Christy Magcalas
Doctoral Student, Psychology

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Christy M. Magcalas
Candidate
Psychology
Department

This dissertation is approved, and it is acceptable in quality and form for publication:

*Approved by the Dissertation Committee:*

Dr. Derek Hamilton, Chairperson

Dr. Daniel Savage

Dr. Benjamin Clark

Dr. Nathan Pentkowski
MODERATE PRENATAL ETHANOL EXPOSURE, SEX, AND HOUSING MODIFY SPATIAL NAVIGATION BEHAVIOR AND HIPPOCAMPAL CELL FIRING IN ADULT RODENTS

by

CHRISTY M. MAGCALAS

M.S., Psychology, University of New Mexico, 2015
B.A., Psychology, University of Nevada, Las Vegas, 2012

DISSERTATION

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For my parents, Deogracias and Antonina Magcalas, and my siblings, both biological and in-law, Caroline, Matt, Cheryl, Steve, Philip, and Lucy. In memory of my sister, Cheryl.
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Prenatal alcohol exposure (PAE) is associated with structural and physiological changes that impact the central nervous system and can result in persistent negative consequences in a broad spectrum of cognitive and behavioral domains including deficits in spatial learning and memory, social behavior, and behavioral flexibility. Previous studies have characterized the influence of PAE on spatial navigation and behavioral flexibility through various behavioral paradigms including the Morris water task (MWT). The current study focuses on examining the behavioral consequences of PAE on directional and place navigation through the use of the MWT. In order to assess directional and place navigation the animals were tested in a 2-day hidden platform protocol and a 2-day cued platform protocol with a 2 week time period between protocols. Day 1 of the hidden protocol consisted of 12 training trials and 1 pool shift test. Day 2 consisted of 8 training trials, 1 pool shift test (opposite of the first test), 4 training trials, and 1 probe trial. The pool shift test consisted of moving the pool to a secondary position. The platform either moves with the pool to a relative location in the pool (directional navigation) or stays in the absolute location in the room (place navigation). Following a 2-
week period the rats were tested in a cued variant of the MWT. The cued variant followed the same scheme as the hidden variant except that there were 8 preferred reversal trials added to the end of day 2. The reversal paradigm involved shifting the pool while the platform was placed in the opposite of the location that the animal showed preference to. There were no significant differences between PAE treatment or sexes in the hidden or cued training trials. In the hidden variant there was a significant interaction in the females between preference for the directional or place location and PAE. Most groups had a preference for the directional platform location or had no preference, while PAE mixed housed female rats displayed a preference for the place location. The behavioral outcomes do not exactly match those of the in-vivo electrophysiology research. Freely, moving in-vivo electrophysiology measures were acquired in a dry land version of the shift manipulation. These recordings showed a significant sex X treatment X housing interaction which was driven by a decreased correlation in the mixed-housed Sac males. Overall, the mixed-housed PAE females show differential spatial navigation behavior in the MWT and the mixed-housed Sac males show a decreased hippocampal cell correlation through the dry land version of the shift manipulation. These outcomes suggest that animals may have distinct search patterns that are sex and housing specific and can be influenced by PAE, but these behavioral changes are not necessarily due to alterations in hippocampal firing.
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CHAPTER 1

Specific Aims

Prenatal alcohol exposure (PAE) is associated with structural and physiological changes that impact the central nervous system[1]. PAE can result in persistent negative consequences in a broad spectrum of cognitive and behavioral domains including deficits in motor behavior, social behavior, and behavioral flexibility. Human studies have revealed that children with Fetal Alcohol Syndrome display impaired spatial learning in the hidden-platform variant of the virtual Morris water task (MWT)[2]. Similarly, rodents exposed to high doses of ethanol during gestation (approximately 200-300 mg/dl) display acquisition deficits in the MWT[3-6]. Rodents repeatedly exposed to moderate levels of ethanol during gestation (approximately 60-80 mg/dl) are spared from acquisition impairments but exhibit long-term memory deficits when tested several days after initial training[7, 8]. The behavioral consequences of moderate levels of ethanol in utero tend to be undetectable under basic behavioral assessments owing to the subtle nature of these effects, however, understanding these effects and their neurobiological underpinnings is critically important for understanding mechanisms and pursuing treatments for fetal alcohol spectrum disorders (FASD). Several studies have dissociated directional navigation, responding towards a certain direction in the environment based on visual information, and place navigation, responding to an absolute location in the environment based visual information[9]. Typically developing humans and rodents prefer to use directional responding when solving dry mazes and multiple MWT variants[10-13]. Moderate PAE consistently spares spatial learning in the MWT and preliminary data has shown that moderate PAE results in sex specific alterations in spatial search behavior. The proposed research is aimed towards understanding alterations in the use of
exploratory and goal-directed spatial strategies (directional vs. place responding) in a rodent model of PAE and the underlying neural mechanisms at the systems level. Frames of reference are a central conceptual aspect of this work. In the MWT, directional responding involves control of behavior by a distal cue frame of reference for orientation and a local frame of reference (the pool apparatus) for localization of a goal, whereas place navigation involves control of spatial behavior by the distal reference frame to guide spatial localization[13]. Local apparatus and distal cue frames of reference also differentially influence neural representations of space realized in the activity of hippocampal place-cells (HPCs; neurons that increase firing when an animal occupies a particular location), which preferentially represent locations in local reference frames with a smaller population of HPCs representing locations defined by distal reference frames[14-19]. Collectively, these findings motivated the central hypothesis that moderate PAE induces alterations in spatial navigation strategies, which are related to alterations in neural representations of spatial reference frames.

**Specific Aim #1: Test the hypothesis that moderate PAE and housing alter spatial navigation behavior in male and female rodents.**

- Experiment 1: Quantify place and directional navigation behavior in male and female rats exposed to moderate levels of ethanol during gestational development. Data will be acquired in a modified version of the Morris Water Task where the animals will be trained to go to a goal location and then assessed in a shift manipulation.

**Specific Aim #2: Test the hypothesis that moderate PAE and housing alter neural representation of frames of references in adult male and female rodents during an exploratory spatial task.**
• Experiment 1: Quantify the representations of local and distal reference frames in populations of hippocampal place cells in male and female rats exposed to moderate levels of ethanol or saccharin during gestation. Data will be acquired during an exploratory spatial task and the predictive value of neural representations and spatial navigation preference will be assessed. If a rat has a preference for place navigation in the Morris water task then its hippocampal place cells will be bound to the distal reference frame and if a rat has a preference for directional navigation in the Morris water task then its hippocampal place cells will be bound to the local reference frame. Based on previous research, most rats should display a preference for directional navigation and have more cells bound to the local reference frame.

Potential Impact: This study provides a better understanding of subtle spatial cognitive deficits in FASDs and insights into the mechanisms involved in altered spatial cognitive due to prenatal alcohol exposure. These experiments are not represented in the field yet and are important for understanding cognitive deficits found in FASDs. Most importantly, acquiring a systems level understanding could support novel treatments specific to FASDs.
CHAPTER 2
Introduction

Exposure to alcohol during gestational development can result in aberrant, long-term structural, functional, and behavioral alterations [1, 20, 21]. The physical dysmorphologies and cognitive consequences of being exposed to alcohol in utero are termed as Fetal Alcohol Spectrum Disorders (FASD), which is an umbrella term inclusive of fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS), alcohol related birth deficits (ARBD), and alcohol related neurodevelopmental disorder (ARND) [22, 23]. FASD is a serious, world-wide public health concern that has substantial economic impact and results in spending hundreds of millions of dollars in annual medical care, special education, and disability services [24-31]. Although FAS is the most known consequence of prenatal alcohol exposure (PAE) most children exposed to alcohol during gestational development have alcohol-related neurodevelopmental problems, but lack the characteristic facial dysmorphology, thus are more difficult to identify [32, 33].

Fetal Alcohol Spectrum Disorders:

Exposure to alcohol during gestational development can result in aberrant, long-term structural, functional, and behavioral alterations [1, 20, 21]. The physical dysmorphology and cognitive consequences of being exposed to alcohol in utero are termed as Fetal Alcohol Spectrum Disorders (FASD), which is an umbrella term inclusive of fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS), alcohol related neurodevelopmental disorder (ARND), and alcohol related birth defects (ARBD) [22, 23]. FAS was first described in 1973 by Jones and colleagues and is the most severe outcome of prenatal alcohol exposure (PAE). FAS is
characterized by facial dysmorphology, including a thin upper lip, a smooth philtrum, and short palpebral fissures, brain and body growth deficiencies, behavioral abnormalities, and structural or functional neurocognitive deficits. **Partial FAS**, which is not as severe as FAS, is characterized by the typical FAS facial dysmorphology, height and/or weight growth retardation, and either altered brain growth/morphology or behavioral/cognitive deficits. **ARND** is defined as having neurocognitive, behavioral, or altered brain growth or morphology due to PAE. **ARBD** involves minor abnormal facial features as described above and structural deficits of at least one organ system [22, 23, 33, 43-45]. Although FAS is often most commonly associated with PAE most children exposed to alcohol during gestation have alcohol related neurodevelopmental problems, but lack the facial dysmorphology, thus are more difficult to identify [32, 33]. The severity of outcomes is dependent upon the amount, frequency, and timing of alcohol consumed by the mother. Other factors that should be taken into consideration are maternal stress, environmental factors, and maternal health and nutrition.

FASD is 100% preventable but affects thousands of children around the world. If FASDs could theoretically be a problem of the past, then why is it the leading cause of mental retardation? One major reason why FASD continues to occur is because of the increasing number of unexpected pregnancies and the large number of women of child-bearing age who consume alcohol. For example, in the United States during 2001-2008, over half of all pregnancies were unplanned [46]. Additionally, the prevalence of drinking in women of child-bearing age was 51.5% in 2006-2010 [47]. and the rate of women drinking, especially binge and heavy drinking, continues to increase [48]. The overlap of these two demographics results in some women consuming alcohol while they are unknowingly pregnant. Some may believe that FASDs is not a major public health problem, but empirical evidence shows otherwise. The risk
of FASD is the leading cause of mental retardation and occurs in multiple countries and maternal of varying socioeconomic statuses, IQ, and ethnic backgrounds [33, 49]. The estimates for FAS, the most severe outcome of PAE, only take part of the spectrum into consideration. As shown in Table 1, FAS prevalence is multiple folds lower than the estimates for FASDs. These estimates are considered to be conservative due to the difficulties in identifying and diagnosing FASD. More importantly, these estimates support that fact that FASD is a serious, world-wide public health concern that has substantial economic impact and results in spending hundreds of millions of dollars in annual medical care, special education, and disability services [25-31, 50].

**FASD Spatial Cognition:**

Prenatal alcohol exposure has been shown to adversely affect spatial cognition in humans and non-human mammals. There are currently no treatments that alleviate the learning and memory deficits in FASD, which may be due to the incomplete understanding of these cognitive alterations. Gaining a comprehensive understanding of the underlying mechanisms involved in FASD related learning and memory deficits are vital for developing potential treatments. Coles et al. (2010) investigated learning and memory abilities in young adults prenatally exposed to alcohol through a nonverbal selective reminding task [51]. In this task, participants were show boxes with different configurations of dots. Each configuration had a target dot and the participants were asked to recall the target dot for each configuration. PAE participants had a decreased rate of learning compared to non-exposed participants, which was evident in the decreased percent of correct choices throughout the learning trials. Although this is not explicitly defined as a spatial learning task, the participants were required to use spatial cognition in order to identify and recall the target within a specific configuration of identical dots. Uecker and
Nadel (1996) identified spatial deficits in children with FAS [52]. In this study, Uecker and Nadel observed that children with FAS were deficient in arranging the objects in their original locations and distorted the spatial distribution of the objects. This task required participants to recall 16 objects and where they were located on a table. Children with FAS were able to recall the objects, but had deficits in recalling where the objects were located on the table and distributed the items in a different configuration than they were presented in. Also, children with FAS were unable to replicate the spatial dimensions of a clock face, which was evident in the unequal distribution of numbers around the clock face. In an additional study, Uecker and Nadel (1998) found that children with FAS displayed spatial deficits when participants were asked to recall the spatial location of pictures in a book [53]. Again, the children with FAS identified fewer correct locations than the control participants. Deficits in spatial organization and visuospatial abilities have also been identified in children with prenatal alcohol exposure even without official FASD diagnoses [32, 54, 55]. Furthermore, Hamilton et al. (2003) tested adolescent males with FAS in a virtual version of the MWT [2]. Participants were tested in 20 hidden platform trials to assess spatial learning, 1 no platform probe trial to assess search strategies, and 8 cued trials to assess the ability to navigate to a visible goal location. Although all participants had similar performance in the initial block (4 trials) of training, FAS participants were deficient in learning the hidden platform location in the subsequent hidden platform trials. This deficit was evident by the increased path lengths to the platform location. Additionally, the probe trial showed that FAS participants had significantly less time in the goal location quadrant than the control participants. Deficits were not due to the participants not understanding or not being able to complete the task because all participants were able to successfully navigate to the goal in the cued trials. All of these studies suggest that PAE induces spatial learning and memory
deficits. Most of these studies focus on learning and memory abilities in children that are still developing their cognitive abilities. Understandably, most of the FASD population has been identified in the past 30 years, but studies on a larger population of adolescents and adults would be incredibly beneficial for understanding how FASD develops past childhood. Also, it is important considering that many animal models utilize testing after maturation.

Models of FASD:

It is important to understand the mechanisms involved in the cognitive and behavioral consequences of PAE. Animal models involving PAE are pivotal for understanding these effects and their neurobiological underpinnings and are critically important for understanding mechanisms for pursuing treatments for FASD. Given that animal models are vital for the progression of understanding and developing treatments for FASD it is also vital to understand how species compare to one another. Cudd (2005) comprised review comparing brain development across humans, primates, rodents, other non-human mammals, and nonmammalian for the purposes of studying alcohol teratology [56]. Different species are considered to be the best option depending on what experimental questions are being addressed. For examples, rats and mice are the most common species used in FASD models because PAE induced behavioral and neuronal consequences shown in rats is similar to those found in the human FASD population. Also, the developmental timelines of rats and mice have been thoroughly researched and are easily comparable to the human developmental timeline. The \textit{in utero} gestational period for rats and mice is equivalent to the first 2 trimesters of human gestation and early postnatal days in rats and mice are equivalent to the human third trimester of gestation. Using rats and mice allows for targeted exposure to specific developmental time points that are translatable to
human exposure. Meanwhile, guinea pigs have a longer gestational period (~68 days), which is beneficial if third trimester maternal drinking effects are the focus of the study. Although guinea pig development is not as directly translational to humans as rats or mice. These are some of the considerations that should be kept in mind when comparing between animal models and when translating animal models to human FASD.

**Morris Water Task (MWT):**

The Morris water task [57] is one of the most widely used behavioral tasks to assess spatial navigation learning and memory abilities. In the MWT, an animal is placed into a circular pool of water that has been made opaque by the addition of milk or non-toxic paint. Distal cues are placed around the testing area to assist with navigation. In the typical variant of the MWT, the animal is released from different start locations and allowed a specific amount of time to find a goal location. The most common variables acquired during each trial are the time (latency) and distance travelled (path length) to find the goal location. Different variants of the task used to assess spatial cognition abilities are dependent upon the type of platform used (hidden, cued, or probe) and the location of the goal location (moving, random, or reversal). The variants of the MWT discussed here are described in further detail for the purposes of this review and are not limited to the following conditions. *Hidden platform* trials are conducted in order to examine spatial learning acquisition abilities and can be used to assess *long-term memory (LTM)*. In this variant the platform is submerged ~1-2 cm below the surface of the water so that the animal cannot see the goal location. The goal location can only be determined by the use of distal cues and distance from the pool wall. Acquisition is typically assessed through giving animals multiple trials with a stationary platform location. The use of a hidden platform in a stationary
location is often referred to as place learning. Normal animals rapidly learn to navigate to the stationary goal location over a few trials [57], which is evident by the direct swim path to the goal location. Long-term memory can be assessed by testing animals in the initial goal location after a period of time following acquisition. *Cued platform*, or visual, trials are conducted in order to examine the animals ability to use a local cue in addition to distal cues to navigate to the goal location. In the cued variant the top of the platform is higher than the surface of the water, thus visible to the animal. Normal animals also rapidly learn to navigate to the goal location at a quicker rate than the stationary hidden platform variant [57]. Both the hidden and cued variants can utilize a stationary method where the platform stays in one location throughout the experiment or a *moving platform* method where the platform is moved to a new location between days or trials. Moving the platform between days can be used as an assessment of short-term memory because the animal has to learn to new platform location every day. When the platform is moved each trial, it is generally referred to as *random* and is used to examine swimming abilities because the platform location cannot be predicted [58]. No platform *probe* trials typically take place towards the end of an experiment once the animal has sufficient acquisition performance that is indicative of and animal learning where the goal location is. In this type of trial the platform is removed from the apparatus and searching variables are acquired. Some variables typically examined are the amount of time spent in each of the quadrants and number of times the animal crosses the learned goal location. Multiple consecutive probe trials can be used to determine extinction behavior and probe trials conducted an extended period of time after learning acquisition can be used to assess long-term memory. *Reversal* trials are utilized to examine flexible behavior and new learning. Reversal trials involve moving the
platform to a new location in the pool, typically in the opposite quadrant of the initial platform location.

**PAE and Spatial Navigation:**

Spatial learning and memory deficits have been identified in multiple models of FASDs. Table 2 summarizes all of the articles referenced here. The learning deficits identified by Hamilton et al. [2] have been replicated in a host of animal models. The similarities of these findings help validate the animal models and provide insight regarding dose and timing effects of ethanol exposure. Mostly young animals display PAE induced spatial navigation acquisition deficits, which were generally not found in older adult animals. These deficits were due to exposure to alcohol throughout most or all of the gestational period or exposure during the early postnatal period. *Chronic exposure throughout (most) of gestation:* Blanchard et al. [59] exposed pregnant dams to a liquid diet containing 35% ethanol derived calories and tested the offspring beginning on postnatal day (PD) 22. PAE animals were impaired in spatial acquisition after the initial day of training. Zimmerberg and Weston (2002) [60] also exposed pregnant rat dams to the same liquid diet as Blanchard and colleagues during gestational days (GD) 6-19 and tested the offspring starting on PD 22. Again, PAE animals displayed longer latencies to find the hidden platform location during acquisition. Gabriel and colleagues (2002) [61] and Christie et al. (2005) [62] exposed rat dams to a similar liquid diet throughout the entire gestational period and found that adolescent offspring continued to display acquisition deficits. Similarly, young mice and guinea pig offspring exposed to high doses ethanol throughout most of the gestational period through gavage or oral administration displayed learning acquisition deficits in the hidden platform MWT variant [63-67]. When reported, these exposures resulted in high maternal BAC
of approximately 300-400 mg/dl. This suggests that heavy exposure to ethanol throughout gestation can result in dysfunctional spatial learning capabilities in young offspring.

Several studies exposing animals to ethanol during the *early postnatal growth spurt* period also demonstrated impaired spatial learning in young offspring. Bañuelos et al. (2012) [68], Ryan et al. (2008) [69], Goodlett and Johnson (1997) [70], Twari and Chopra (2011) [71], and Pauli et al. (1995) [72] all demonstrated that exposure to high levels of ethanol via gavage at some point during the brain growth spurt period (GD 4-10) leads to high BACs and impaired spatial acquisition of the hidden platform location for young animals (testing began before PD 45). Similarly, Wagner et al. (2014) [3], Goodlett and Peterson (1995) [73], Goodlett et al. (1987) [5], Filgueiras et al. (2010) [74], and Wozniak et al. (2004) [75] found similar acquisition deficits in young animals when exposing early postnatal pups to high levels of ethanol through injections or artificial rearing. Peak BACs as a result of ethanol injections were 240-510 mg/dl and as a result of artificial rearing were 112-408 mg/dl. The most interesting finding was that acquisition deficits were dose dependent. Animals that were exposed to a lower dose of ethanol throughout the early postnatal exposure period had a lower peak BAC and was only slightly impaired. Animals that were exposed to a higher dose in a condensed period had a higher peak BAC and were more severely impaired in the acquisition of the hidden platform location [70]. These findings support that the effects of ethanol are dependent upon the amount consumed and the frequency at which it is consumed.

Spatial learning deficits are not limited to childhood and adolescent time periods. Many studies have found that learning may continue to be impaired throughout early-mid adulthood (> 3 months of age). Gianoulakis (1990) [6] exposed pregnant rat dams to a liquid diet containing 36% ethanol derived calories throughout the entire gestational period. PAE offspring tested at
40, 60 and 90 days of age all showed deficits in the hidden platform variant of the MWT. The learning acquisition deficits were most evident at the PD 40 and 60 time points. At the PD 90 time point, the PAE animals were able to sufficiently acquire the task after the initial trial, but were impaired on the first trial of each day. Many studies involving heavy ethanol exposure throughout gestation resulted in disrupted spatial learning [76-79]. Additionally, spatial learning can be negatively impacted by heavy ethanol exposure during early postnatal days. In a study conducted by Johnson and Goodlett (2002) [4] there was a significant effect of heavy early postnatal ethanol exposure on hidden platform place learning when animals were exposed during PD 7-9. Similarly, Thomas et al. (2007) [80] and (2008) [81] demonstrated that heavy exposure to ethanol during the early postnatal period results in spatial learning deficits in adolescence (PD 52) and persists through adulthood (~5 months of age).

Although some studies show that adult animals exposed to ethanol during gestation have impaired acquisition abilities there is still controversy over whether or not this is true. A number of studies have found a lack of spatial learning in adulthood. For example, Wozniak et al. (2004) [75] injected mice pups on PD 7, which generated a peak BAC of 510 mg/dl. When the animals were tested at a young age (PD 28 or 2.5 months of age) the PAE mice displayed an impaired ability to learn the hidden platform. When the mice were retested after maturation (5-6 months or 8 months respectively) all of the PAE animals learned at a similar rate as the control animals except for one block of trials. The only difference in the mature PAE animals was an increase in path length in the 8 month old PAE animals on block 8 of 10. Summers et al. (2006) [82] and Minetti (1996) [83] also utilized a single exposure day (GD8) and found no acquisition deficits in adulthood or in younger animals. When examining the effects of an ethanol exposure throughout gestation Cullen et al. (2014) [84] found that a more moderate dose of ethanol (6 or
15%) in a liquid diet did not result in acquisition deficits in adult (8-10 months) or aged (15-18 months) offspring. Studied conducted by Hamilton et al. (2014) [8] and Sutherland et al. (2000) [85] both used moderate levels of ethanol throughout the entire gestational period and found that adults lacked the spatial acquisition deficits found in heavy ethanol exposures and younger PAE animals. Additionally, in a study conducted by Gabriel et al. (2002) [61] rats prenatally exposed to a liquid diet containing 36% ethanol derived calories showed learning deficits at 2 months of age, but learning deficits were undetectable at 13-14 months of age. Wei et al. (2013) [86], Zink et al. (2011) [87], Thomas et al. (2010) [88], and Byrnes et al. (2004) [89] are among the additional studies that failed to find spatial learning deficits due to PAE. These findings suggest that there may be a threshold for the amount of ethanol needed to induce basic spatial learning deficits. Due to the controversial evidence, it is important to further investigate more complex spatial behaviors, such as flexible learning and overall search behaviors.

One way to distinguish dysfunctional spatial abilities is through observing behavior during no platform probe trials. Examining how long animals persist in the target location after acquisition can provide insight on how well the task was learned. Generally, if the goal location was sufficiently learned animals will search persistently in the learned target location and the close, surrounding area. Banuelos et al. (2012), Ryan et al. (2008), Wagner et al. (2014), Johnson and Goodlett (2002), Goodlett and Johnson (1997), Goodlett et al. (1987), Goodlett and Peterson (1995), Tiwari and Chopra (2011), and Wozniak et al. (2004) all observed negative spatial learning in both hidden platform and probe trials [3-5, 68-71, 73, 75]. These studies all utilized heavy exposure to ethanol during the early postnatal period (PD 4-10). This suggests that heavy exposure late in pregnancy (i.e. human third trimester) could be especially detrimental to offspring. Additionally, Gianoulakis (1990) observed deficits in both initial learning and search
behavior when rats were exposed to ethanol throughout the entire gestational period [6]. This finding adds to the range of exposure paradigms that demonstrate spatial deficits in multiple domains. Even though these studies inform the field on the severity of PAE induced spatial processing deficits it is logical that an animal impaired in sufficiently learning goal location would also fail to search the appropriate area. The studies that only examine deficits in the probe trial while acquisition deficits are spared could be even more informative because they focus more on how a PAE animal completes a spatial task rather than focusing on if an animal can complete the task. However, these findings only occur in a slim number of studies. In a study conducted by Vega et al. (2013) pregnant dams voluntarily consumed 10% ethanol during the first 8 days of gestation [90]. Adult mice offspring were able to learn the hidden platform location sufficiently but took longer to enter the target area during the probe test. Interestingly, the PAE animals persisted in the target region longer than the control animals during the probe test. Zink et al. (2011) also found differences in the performance of the PAE animals during the probe test only [87]. The rats were exposed to vapor chambers in utero through postnatal day 8. This study is particularly interesting because it is one of the only rat or mice studies that expose rats throughout in utero gestation and during the early postnatal period. These animals were spared acquisition deficits but had longer latencies to enter the learned goal location. These two studies may have found these specific effects because they used a moderate exposure compared to a heavy exposure, but these conclusions are speculation due to the fact that Zink and colleagues never reported the exact amount of ethanol they used. Instead, the ethanol levels reported here were estimated on a previous study that they referenced [91].

Testing animals in a moving hidden platform variant is a more difficult task compared to the stationary hidden platform variant. In this variant, animals are required to learn a new
platform location every day. This means that the information that had been learned previously will not predict the new information that needs to be learned. It has been shown that children with FASD are able to solve simple tasks, but have a difficult time solving more complex tasks [35]. Similarly, Sutherland et al. (2000), Savage et al. (2002), and Shea et al. (2012) found that exposure to moderate levels of ethanol throughout gestation leads to deficits in the moving platform variant of the MWT [85, 92, 93]. Spatial deficits have also been identified in heavy exposure models [58, 88, 94]. Reversal learning also requires new learning but takes place after a stationary goal location has been well established. Young PAE animals have displayed increased latency to find the “reversed” platform location [61] and adult animals have displayed increased perseveration errors to the old location [8]. Generally, PAE animals are capable of learning the reversed goal location suggesting that the moving platform variant is more difficult than the reversal variant [59, 64, 78]. In contrast, the cued variant of the MWT is considered to be the easiest task because there is a visible local cue in the goal location. Behavior in the cued variant did not differentiate FAS participants from control participants as shown by Hamilton and colleagues (2003). Most studies did not find any differences in performance on the cued variant between PAE and control animals. The random variant performance utilized to assess swimming ability was not affected by PAE. This indicates that differences in the water task are not due to PAE induced swimming deficits.

**Dry land mazes:**

Dry land mazes are also often used to assess spatial cognition but are contingent upon motivation to acquire a food reward at the goal location. In order to motivate animals to direct its behavior towards a reward, the animals are often placed on a restricted diet opposed to the MWT
where there is intrinsic motivation to get out of the pool of water. Never the less dry land maze assessments allow the field to examine spatial navigation behaviors outside of the water task. These tasks can also be used in conjunction with freely moving in vivo recordings and local infusion experiments without being compromised by possible water damage. Many studies have identified spatial navigation differences due to PAE in various dry land tasks. For example, Popović et al. (2006) utilized an unconventional can test to assess the effects of a liquid diet with ethanol on spatial discrimination abilities [95]. The adult offspring of dams that consumed more moderate levels of ethanol throughout the gestational period (peak BACs ~104 mg/dl) and/or throughout the early postnatal period (peak BACs ~ 107 mg/dl) were trained to discriminate the goal can from a set of identical cans. PAE offspring displayed impaired learning in this task, which were most evident in offspring that were exposed to ethanol during the early postnatal period. This test is relatively simple and yet the continuous exposure to ethanol resulted in spatial learning deficits. In another task, the Y-maze, animals are given a reward at one end (sample arm) while the other arm is closed off (choice arm). During the testing phase, the animal must learn to select the choice arm. On the next test, the sample location and choice locations are switched. This alternation task requires the animal to remember which arm was baited previously and understand that the reward will be in the opposite arm. This task is also used in the T-maze, which is nearly identical to the Y-maze except that the shape is configured to a “T” shape instead of a “Y” shape. Interestingly, guinea pigs exposed to high levels of ethanol throughout gestation are not impaired in the Y-maze alternation task [96] while rats exposed to heavy levels of ethanol throughout gestation or during the early postnatal period had impaired acquisition in the T-maze alternation task [88, 97]. Although Dobson et al. (2012) was unable to identify treatment differences in the Y-maze, they did observe an increased number of errors in the Biel-maze while
acquisition deficits were spared. Similar to the task completed in the Y and T-mazes, the delay non-match to place task tests the ability to choose a novel choice location that is spatially separated from the initial sample location. Brady et al. (2012) observed decreased correct arm choices in PAE young adult mice, but Kim et al. (1997) did not find delay non-match to place deficits in older PAE rats (4-12 months old). These results are particularly interesting because this is one of the few times where deficits are identified in the moderately exposed animals (BACs ~88 mg/dl) and spared in the animals exposed to heaver doses of ethanol (BACs ~150 mg/dl) especially considering that both sets of animals were exposed throughout gestation. Finally, PAE deficits have also been identified in the radial arm maze. In one variant of the radial arm maze, animals must learn to enter each individual arm for a food reward. Entering an arm repeatedly is considered to be an error. A single exposure to a high dose of ethanol during the brain growth spurt time period [75] and exposure to ethanol in utero [54, 99] have been shown to induce deficits in the radial arm maze. These dry land mazes are particularly important for showing how PAE negatively affects spatial working memory abilities.

**Sex differences:**

Sex effects are not always assessed in spatial navigation or developmental alcohol exposure studies. Few human FASD studies assess sex differences within the FASD population, which may be due to the relatively low numbers of subjects used in FASD research. Even so, a few studies have been able to identify sex differences in the FASD population. For example, Rasmussen et al. (2006) identified that girls displayed more severe executive functions deficits compared to males [100]. Also, Tesche et al. (2015) reported FASD sex differences in hippocampal, temporal cortex, and auditory cortex MEG activation during the odd-ball task.
Contrary to human studies, animal models have identified sex dependent effects in PAE animals. Blanchard et al. (1987) exposed pregnant dams to a liquid diet containing 35% ethanol derived calories and tested the offspring beginning on postnatal day (PD) 22 [59]. PAE females were impaired on the hidden platform variant of the MWT for days 2 and 3 (out of 3 total days) while PAE males were only impaired on day 2. This suggests that females were more impaired than males by PAE and that PAE males are able to acquire spatial learning, just at a slower rate. Interestingly, during the first reversal trial, PAE males showed less perseveration errors to the original location than females and other treatment groups. Kelly et al. (1988) exposed rats to moderate and high doses of ethanol during the early postnatal period and found that spatial acquisition was only impaired in adult female with heavy PAE exposure [102]. Several additional studies have identified spatial navigation deficits that are more severe in female PAE offspring compared to male PAE offspring [3, 68, 69, 80, 82, 83]. In contrast, a few studies have also identified more severe spatial deficits in PAE males compared to females. For example, in a study conducted by Johnson and Goodlett [4] hidden platform place training and probe test deficits and were more severe in male PAE rats than female PAE rats. These results built upon and earlier study that identified exaggerated PAE related spatial deficits in males exposed to high doses of ethanol during PD 7-9 compared to females [73]. Sex-differences in PAE animals have been identified in a number of studies, but even more studies have found a lack of sex-differences. Also, there are multiple studies that used both males and females but did not address sex as a factor [65, 66, 75, 88, 90, 93, 103]. Contradicting results suggests that there is a need for additional FASD studies to examine the effects of sex in conjunction with PAE. There is a particularly dire need to examine sex differences in humans with FASD at multiple time points...
(childhood, adolescence, and adulthood) to understand how PAE differently affects cognition throughout development.

**Neural Mechanisms:**

As discussed, there are significant spatial behavioral consequences associated with FASD and PAE, but what are the mechanisms behind these dysfunctions? Alterations to hippocampal structures may begin to explain the dysfunctional spatial behavior found in humans with FASD and PAE animals. First, it is important to understand the vital role the hippocampus has in spatial navigation. For decades, the hippocampus has been identified as playing a key role in storing and processing spatial information. For example, O’Keefe and Nadel (1976) suggested that the hippocampus served as a cognitive map where spatial information is stored, which was based on Edward Tolman’s cognitive map theory [104]. The case of H.M. further supported the importance of the hippocampus in spatial learning. Patient H.M. had bilateral temporal lesions to help alleviate epileptic seizures and most of his hippocampus was obliterated. H.M. experienced episodic memory loss and major deficits in spatial acquisition [105]. Morris et al. (1982) was among the first to identified the connection between hippocampal lesions and spatial acquisition performance in the MWT [106]. Rats that received total (or near total) hippocampal damage displayed severe learning deficits in hidden platform place learning. Since then, a vast amount of experiments have expanded upon the importance of the hippocampus in spatial navigation [9, 107-109]. Spatial deficits found in FASD humans and animal models are similar to spatial deficits found in hippocampal damaged human and animal studies. These similarities suggest that the teratogenic effects of alcohol may target the hippocampus. Uecker and Nadel (1996) [52] stated that, “the children with FAS performed almost identical to patients that had
undergone right temporal lobectomy with large lesions to the hippocampus” referring to a study conducted by Smith and Milner (1981). Overall decreased brain volume and altered hippocampal volume and activation have been identified in the FASD population [94, 101, 111]. More specifically, Miki et al. (2004) identified decreased CA2/3 volume and an overall decrease in pyramidal cells in young rat pups exposed to high levels of ethanol during the early postnatal period [112]. Bonthius and West (1991) observed a decreased in the number of pyramidal cells in the CA1 field in rat pups exposed to heavy levels of ethanol throughout PD 4-11 [113]. Gonzales-Burgos et al. (2006) also reported a decrease in the number of pyramidal cells in the CA1 field in PAE offspring [114]. Interestingly, these findings were the result of a moderate-heavy exposure throughout gestation to a liquid diet containing 35% ethanol derived calories (BAC ~111 mg/dl). Several additional studies also reported that heavy exposure to ethanol during PD 4-10 [115], throughout part of gestation (GD 10-21, [116]), and throughout most of gestation (GD 6-21, [117]) results in decreased pyramidal cells in the CA1 field of the hippocampus. Ethanol exposure during gestation and during the third trimester equivalent time frame can result in negative hippocampal structural alterations. Despite the fact that these studies are informative, the effects of low-moderate exposures have yet to be identified.

The previously discussed research has identified hippocampal neuronal loss due to heavy ethanol exposure, but do PAE animals experience loss in hippocampal function? In vivo electrophysiology have been conducted in multiple studies to examine the effects of PAE on long-term potentiation (LTP) and synaptic plasticity. LTP and plasticity are often linked with learning abilities. Optimal LTP and plasticity conditions are required in order for sufficient learning to occur. For example, blocking LTP impairs learning and acquisition in the MWT [118], while a saturation of LTP also disrupts learning [119, 120]. Exposure to high doses of
ethanol throughout gestation results in decreased hippocampal LTP in PAE offspring [62, 64, 94, 121]. Sutherland et al. [122] exposed pregnant dams to moderate levels of ethanol in a liquid diet throughout gestation, which produced peak BACs of 83 mg/dl. Rat pups were anesthetized with urethane while in vivo electrophysiology measures were acquired. Sutherland and colleagues reported decreased LTP in the dentate gyrus of the hippocampus of PAE offspring, while baseline evoke responses were the same for all treatment groups. These results have been found in addition studies and support that moderate levels of ethanol also produce adverse alterations in hippocampal LTP and plasticity [123, 124]. Interestingly, sex differences have been identified in PAE related LTP alterations. Titterness and Christie (2012) identified decreased LTP in PAE males and enhanced LTP in PAE females after moderate-high ethanol exposure (BACs of 87-115 mg/dl) [125]. This suggests that both males and females need to be included in additional studies in order to expand upon sex-dependent effects of PAE.

Unfortunately, the mechanisms underlying the cognitive deficits associated with FASD are currently not well understood. It is of particular importance to gain a better understanding of the cognitive deficits associated with the less severe FASD and their respective neurological mechanisms considering that they make up the vast majority of FASD cases in the world. Among the multiple behavioral and cognitive PAE related changes, alcohol exposure during gestational development can lead to persistent learning and memory deficits [1, 2, 21, 34-39]. Children with FAS display impaired spatial learning in the hidden-platform variant of the virtual Morris water task [2]. Similarly, rodent offspring whose mothers exhibited high peak blood ethanol concentrations (~200-300 mg/dl) also display acquisition deficits in the hidden-platform variant of the Morris water task (MWT) [3-6].
There are currently no treatments that alleviate the learning and memory deficits in FASD, which may be due to the incomplete understanding of these cognitive alterations. Gaining a comprehensive understanding of the underlying mechanisms involved in FASD-related learning and memory deficits are vital for developing potential treatments. Studies have found that rodents repeatedly exposed to moderate levels of ethanol during gestation (approximately 60-80 mg/dl) are spared from impairments in acquisition but exhibit long-term memory deficits when tested several days after initial training [7, 8]. The behavioral consequences of moderate levels of ethanol in utero tend to be undetectable under basic behavioral assessments owing to the subtle nature of these effects. However, understanding these effects and their neurobiological underpinnings is critically important for understanding mechanisms and pursuing treatments for FASD.

Several studies have dissociated directional navigation, responding towards a certain direction in the water maze environment based on visual information, and place navigation, responding to a place location in the environment based visual information [9]. Many of these studies assessing spatial behavior assume that animals use place navigation to determine the goal location in the MWT. Place navigation involves localizing the goal location based on its relation to the fixed cues outside of the pool walls (e.g. distal cues) [48]. This assumption is based on the fact that the only unique visual cues are located outside of the pool wall, which leads some to believe that rodents triangulate where the goal location is in relation to how close or far it is from the different distal cues [49,50]. In contract to place navigation, directional navigation uses visual information to determine orientation and navigate accordingly [51]. In directional navigation, an animal learns how to navigate to the goal location by taking a consistent route towards a goal. Distal visual information is used to determine which direction to orient to and the final goal location is determined by local visual information (e.g. distance from the pool wall). Many studies
from our laboratory and others have assessed navigational behavior in order to determine if animals are actually using place navigation or another type of navigation. These studies have found that typically-developing humans and rodents actually prefer to use directional responding when solving dry mazes and multiple MWT variants [10-13]. Moderate PAE consistently spares spatial learning in the MWT, however, whether moderate PAE alters spatial strategies has not been determined. To address this question, the effects of moderate PAE or saccharin (control) exposure on preference for directional or place responding in the MWT were evaluated. Previous studies were limited to male rats that were assessed in the cued platform variant of the MWT [40-42]. This study expands upon previous research conducted in our laboratory to study the influence of sex by including female rats. A pair housing manipulation was also included in the study which allows for the examination of social influence on spatial behavior.
CHAPTER 3
Methodology

All procedures included adhere to the Public Health Service policy on humane care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of New Mexico Health Sciences Center and main campus.

Moderate Prenatal Alcohol Exposure:

The moderate PAE protocol has been established and extensively used in our laboratories over the past decade [7, 8, 126, 127]. Female Long-Evans rats (Harlan Industries, Indianapolis, IN), 70 to 90 days of age, were shipped to the University of New Mexico HSC Animal Research Facility (ARF) where they were maintained on a reverse light-dark cycle (lights on 2100 – 0900 hours) and provided rat chow and water ad libitum at all times. After one week of acclimation to the facility, these females were allowed daily four-hour (1000 – 1400 hours) access to increasing concentrations ethanol in 0.066% saccharin water. Specifically, rats received 0% ethanol on days 1-2, 2.5% ethanol on days 3-4, and 5% ethanol for the remainder of a two-week period. At the end of the two-week pre-pregnancy drinking period, the daily average ethanol consumption was determined for each female and all rats whose ethanol consumption was less than one standard deviation below the mean of the group was removed from the study. The remaining female rats were randomly assigned to either a saccharin control (SAC) or 5% ethanol group (PAE) and were matched to balance the mean pre-pregnancy ethanol consumption between groups. Next, the females were placed with a proven male breeder until pregnancy was confirmed by the presence of a vaginal plug. Rats did not have access to ethanol or saccharin during breeding, which required
an average of 1.5 days. Beginning on gestational day 1, rat dams were given 0.066% saccharin water containing 0% or 5% ethanol according to the treatment assignment. Drinking sessions were equivalent to the pre-pregnancy drinking sessions, in that rats had access to saccharin water with 0% or 5% ethanol for 4-hours every day throughout gestation. The volume of saccharin water was matched to the mean volume of saccharin water consumed by the ethanol-drinking group. Treatment ceased as soon as the pups were born.

Consumption and BACs:

Blood alcohol concentrations (BAC) were acquired from a separate set of dams that did not provide offspring for the behavioral studies to avoid potential stress confounds. Daily 4-hour consumption averages at ~2.04 g/kg, which results in a peak BAC of ~61 mg/dL [126, 128, 129]. No differences in maternal weight gain or offspring-birth weight were observed.

Housing:

Litters were weighed, culled to ~10 pups, and weaned on postnatal day 24. At 3-4 months of age, pair-housed offspring were transferred to the Psychology Animal Research Facility where they were allowed to acclimate for 2 weeks. In order to avoid possible litter effects, rats were randomly sampled so that no more than 2 rats from each combination of litter and sex were included in any experiment. Rats were pair-housed with a rat of the same sex from either the same prenatal treatment group (Same; Sac-Sac or PAE-PAE) or a difference prenatal treatment group (Mixed; Sac-PAE).
Spatial Navigation Assessment in the Morris Water Task (MWT):

Pool Shift – Hidden Variant:

Directional and place navigation strategies were assessed in adult rats (>PD150) in the following sex-treatment-housing combinations: Male-Sac-Same (n=11), Male-Sac-Mixed (n=10), Male-PAE-Same (n=10), Male-PAE-Mixed (n=10), Female-Sac-Same (n=8), Female-Sac-Mixed (n=14), Female-PAE-Same (n=10), Female-PAE-Mixed (n=15). Spatial navigation strategies were measured in the MWT during adulthood (>PD150). Rats were trained to navigate to an escape platform (16cm x 16cm) in a circular pool (1.5m diameter, 46cm high) filled to a depth of ~24cm. The cool water (~22-24°C) was made opaque by the addition of nontoxic white paint. An overhead camera captured MWT sessions and digital video was tracked and analyzed by software developed in our laboratory [40, 42]. On day 1, all rats were given 12 training trials and 1 navigation strategy test. On day 2, rats were given an additional 12 training trials and 1 navigation strategy test (described below). During the training trials, rats were released 3 times in each of the 4 release points. The release points were randomized within and between each block of 4 trials. On day 2, the rats were given 12 training trials where the pool and platform were in the original, training location. For each navigation strategy test the pool was translated by 0.75m (the radius of the pool) in the room and the platform either remained in the same relative location within the pool or remained in the same spatial location in the room (Figure 1). A preference for directional responding would result in a more efficient path to the relative location in the pool. A preference for place navigation would result in a more efficient path to the spatial location of the platform in the room. All rats received both types of navigation strategy tests, one on day 1 and the other on day 2. Tests were balanced such that an equal number of rats from each prenatal treatment group
and sex received the place navigation test first, while the other half received the directional navigation test first.

Pool Shift – Cued Variant and Preferred Reversal:

Previous research conducted in our laboratory has shown that typically developing male rats show a preference for directional navigation behavior in this pool shift variant of the MWT. This research expands upon these data by including developmental ethanol exposure, sex, and housing variables and due to the fact that the platform was hidden during the training trials. In order to more directly compare this new research to our previous findings a subset of animals were tested in an cued variant of the pool shift task that was equivalent to the methods of our laboratory’s previous work. This subset of animals included male and female rats that were exposed to ethanol or saccharin during gestational development but did not include the housing manipulation because it was not in the scope of the research at this time. Housing was not a factor that was observed or assessed at this point in testing. All of the animals included in the cued variant of the MWT were pair-housed with an animal that was exposed to the same prenatal treatment. Because all of the rats were in the same housed condition, housing is not included as a factor at this point. One week following the hidden platform variant of the water task, the animals were trained and tested in the same regiment, but with a visible platform. On day 1, all rats were given 12 training trials and 1 navigation strategy test. On day 2, rats were given an additional 8 training trials, 1 navigation strategy test, 4 training trials, and 8 preferred reversal trials. The reversal paradigm involved shifting the pool while the platform was placed in the opposite of the location that the animal showed preference to. Preferences for a location were determined immediately after the second pool shift test was conducted and were based on how long it took each animal to reach the place
and navigational goal locations during the cued pool shift tests. It was deemed that an animal had a preference for a specific location if they travelled to that location faster than the opposite location. If they took the same amount of time to travel to both cued locations, then preference was based on their performance during the hidden platform pool shift test. Latencies to reach the escape platform were acquired for all training, testing, and reversal trials.

**Microdrives:**

Microdrives were comprised of 4 recording tetrodes and 1 reference electrode which consisted of four-channel electrodes constructed by twisting together strands of 25 micron insulated nichrome wires (Nichrome, formvar insulation, A-M systems) and heating the tetrodes to bind the individual wires into a bundle. The tetrodes were housed inside a hypodermic stainless steel cannula (A-M systems) and each individual electrode was attached to separate pins on a millmax strip and secured with Silver Print II (GC electronics). The drives were protected by epoxy and each were connected to 3 screws with dental cement (Teets Denture Matrial, Co-oral-ite Dental MFG Co.) to allow for the slow insertion of the tips of the tetrodes into the brain tissue. The drives were bubble tested to make sure that all of the connections were stable prior to implantation.

**Surgery and Post-Op Care:**

Adult rats were deeply anesthetized with a mixture of isoflurane (1.5-4 liters/minute) and oxygen (3 liters/minute) and were chronically implanted with microdrives under stereotaxic guidance. The skull was exposed, Bregma and lambda were leveled, and six anchor screws were tapped into the skull, 2 screws each into the frontal, parietal, and occipital bones. A small hole was drilled into the skull and the microdrive was placed ipsilaterally above the right dorsal
hippocampus in order to record from the CA1 pyramidal cell region of the hippocampus (3.5-3.7 mm posterior and 2.5 mm lateral to bregma, Paxinos and Watson, 1986). The dura matter was removed and the tetrodes were lowered into the cortex so that the tips of the tetrodes was submerged into the tissue 0.5-1 mm into the cortex. The tetrodes and exposed cortex were sealed with bone wax the microdrive was attached to the anchor screws and the skull using dental cement so that the tips of the tetrodes could advance vertically through the tissue to the target region. All of the animals were given >1 week of post-operative rest where they received buprenorphine i.p. injections for pain management and antibiotics in order to stave off infection. All animals were housed individually after surgery to ensure proper healing and to allow for a decreased possibility that the implant would be dislodged.

**In-vivo Behavioral Procedures:**

After the post-op recovery period all of the animals were placed in a white, cylinder apparatus (.895m diameter) with a white bottom for screening and recording sessions. Various distal cues were placed around the behavioral room in order to establish a similar environment that was used in the MWT sessions. Two dimmable lights were placed above the apparatus in order to illuminate the recording area sufficiently and evenly. During each screening session, the microdrives were connect to a multi-channel headstage and the animals were allowed to become familiar to the apparatus for 5-10 minutes. At this time fruit cereal was placed around the apparatus in order to encourage exploration. Tetrodes were slowly moved down into the CA1 area of the hippocampus over several screening sessions and recording session began once local field potentials displayed theta-like rhythms. The rats were recorded in the initial location for ~15 minutes until the entire apparatus was sampled. After the initial recording session, the rat was
returned to its home cage and placed back in the colony room for 1-2 hours. The apparatus was shifted in a similar vein as the pool was shifted during the MWT testing sessions. Once the apparatus was moved the distance of the radius, the rat was brought back into the behavioral room and recorded for another ~15 minutes.

**Data Acquisition and Analyses:**

Output from the headstage was passed through a data acquisition system (Cheetal Digital Interface, ERP-27, Cheetah 32, Neuralynx) with 32 channels of analog signal acquisition at 32KHz and were visualized and collected through the Neuralynx Cheetah Data Acquisition Software. The headstage contained red and green LED lights that indicated the rat’s position. This position was tracked and sampled at 30 Hz. Continuous data was saved and processed offline through SpikeSort 3D (Neuralynx) where clusters/cells were identified and isolated. All cells that were identified were included in the analyses, which included principal cells and interneurons. All cells had a minimum peak firing rate of 1 Hz and had at least 100 active spikes. The cells were then split into principal neurons (4-12 Hz) and interneurons (>12 Hz). Visual inspection of the wave forms was also conducted in order to determine if the cluster was indicative of cell activity or noise. All cells were merged with the time-stamped position of the rat. The spikes were binned in 5x5cm bins and divided by the occupancy data in order to produce rate maps. The rate maps were then smoothed and the spike matrices were used for group comparisons. The cells from shift session were correlated to the cells from the original location session in order to determine the stability of the cell throughout the shift manipulation and is presented as a correlation coefficient. Furthermore, a rotational analysis was conducted in order to determine if the cell shifted with the apparatus or stayed relative to the distal cues. One cell was rotated 360 degrees and was compared to the shifted
cell throughout each degree. The degree where the two cells were least alike helped determine if the cell shifted with the apparatus, stayed stable to the distal cues, or remapped. If the cells were the least similar around 360 degrees, then the cells were categorized as being bound to the apparatus and indicative of a place-bound cell. If the cells were least similar around 180 degrees the cells were categorized as being bound to the distal cues and indicative of a directional-bound cell. If the cells were least similar in between the place and directional categories they were deemed to be remapped or undetermined. The correlational coefficients and degree of minimal likeness were quantified and analyzed in an analysis of variance (ANOVAs) with sex, prenatal treatment, and housing as factors. Also, the number of cells in each rotational category were quantified and reported.

All statistics were analyzed through SPSS Statistics (IBM) and/or Jamovi (https://www.jamovi.org/) and all results reported below were significant at p < 0.05 unless noted otherwise. Effect sizes (partial eta squared, \( \eta_p^2 \)) are provided for all significant effects.
CHAPTER 4

Results

Hidden Platform Training:

Latency to the goal location decreased for all groups throughout the 6 blocks of training that took place over 2 days of training and testing (see Figure 2, \( F(1, 80) = 910.93, p < 0.001, \eta^2_p = 0.919 \)). No significant main effects of prenatal treatment (\( F(1, 80) = 2.46, p = 0.121, \eta^2_p = 0.03 \)), sex (\( F(1, 80) = 0.054, p = 0.816, \eta^2_p = 0.001 \)), or housing (\( F(1, 80) = 0.27, p = 0.61, \eta^2_p = 0.003 \)), and no significant interactions between the 3 factors (\( F(1, 80) = 2.01, p = 0.16, \eta^2_p < 0.001 \)), were observed during the training blocks. All groups performed the same throughout the entire training session, which suggests that all animals were able to sufficiently learn where the goal was located. Additionally, all animals learned to take more direct and efficient routes to the goal location over time, which was apparent by the decreased latency throughout the blocks and days (see Figure 2, \( F(5, 400) = 270, p < 0.001, \eta^2_p = 0.771 \)).

Hidden Platform Shift Tests:

The sex X prenatal treatment X housing interaction was not significant in the hidden platform shift tests (\( F(1, 79) = 0.084, p = 0.773, \eta^2_p = 0.001 \)). Both the mixed-housed rats (see Figure 3, \( F(1, 44) = 4.14, p = 0.048, \eta^2_p = 0.086 \)) and the same-housed rats showed a significant main effect of sex (see Figure 3, \( F(1, 35) = 5.26, p = 0.028, \eta^2_p = 0.131 \)). The mixed-housed males took significantly longer to find the goal location than the mixed-housed females, but the same-housed females took longer to find the goal location than all other groups. Also, there was an
overall main effect of housing for the place location \((F(1, 79) = 4.08, p = 0.047, \eta^2_p = 0.049)\) which was not observed for the directional location \((F(1, 79) = 0.885, p = 0.350, \eta^2_p = 0.011)\). The mixed-housed animals took significantly longer to find the place location than the same-housed animals \((F(1, 79) = 4.08, p = 0.047, \eta^2_p = 0.049)\). There was a significant shift test X prenatal treatment X housing multi-variant interaction (see Figure 4, \(F(1, 79) = 5.51, p = 0.021, \eta^2_p = 0.065\)). All animals that were pair-housed with an animal of the same prenatal treatment showed no significant difference in latency for either the directional or place location. All same-housed animals, despite prenatal treatment and sex, took approximately the same amount of time to find both the directional and place locations (see Figure 4, \(F(1, 35) = 1.65, p = 0.207, \eta^2_p = 0.045\)). Although, the mixed-housed animals displayed different behavior for the directional and place locations. The saccharin-exposed, mixed-housed animals displayed similar behavior that was consistent with our previous studies [40-42] in that they found the directional location significantly quicker than the place location (see Figure 4, \(F(1,38) = 4.58, p = 0.039, \eta^2_p = 0.108\)). The PAE animals displayed a significant housing X sex interaction (see Figure 5, \(F(1, 41) = 5.03, p = 0.03, \eta^2_p = 0.109\)) while the saccharin-exposed control animals did not (\(F(1, 38) = 2.97, p = 0.093, \eta^2_p = 0.073\)). The mixed-housed PAE females had an increased latency to the directional location compared to the place location, while the same-housed PAE females did not display a significant difference in latency to either location. Both the mixed- and same-housed PAE males performed similarly when navigating to the directional and place locations. This suggests that only the mixed-housed PAE females had a significant preference for a location.

There was also a significant sex X housing interaction for latency to the directional location (see Figure 6, \(F(1, 79) = 8.12, p = 0.006, \eta^2_p = 0.093\)). Not only did the males perform differently
than the females, but the housing condition altered behavior as well. The same-housed males took longer to swim to the directional location compared to the mixed-housed males while the same-housed females swam significantly quicker to the directional location compared to the mixed-housed females.

A score was calculated for all of the animals to quantify a preference for the directional or place location ((latency to directional location – latency to place location)/(latency to directional location + latency to place location)). A negative value represented a preference for the place location and a positive value represented a preference for the directional location. There was a main effect of housing for this preference score (see Figure 7, F(1, 79) = 6.32, p = 0.014, \( \eta_p^2 = 0.74 \)). Overall, mixed-housed rats had a preference for the directional location (preference score = 0.0841) and the same-housed animals had a preference for the place location (preference score = -0.1253). No significant interactions were observed for the preference score data (sex X housing X treatment (F(1, 79) = 0.064, p = 0.802, \( \eta_p^2 = 0.001 \)), sex X housing (F(1, 79) = 1.387, p = 0.242, \( \eta_p^2 = 0.017 \)), sex X treatment (F(1, 79) = 0.434, p = 0.512, \( \eta_p^2 = 0.005 \)), housing X treatment (F(1, 79) = 2.296, p = 0.134, \( \eta_p^2 = 0.028 \)). No significant main effects of prenatal treatment (F(1, 79) = 0.947, p = 0.334, \( \eta_p^2 = 0.074 \)) and sex (F(1, 79) = 1.732, p = 0.192, \( \eta_p^2 = 0.021 \)) also were not found in the preference score data.

**Cued Platform Training:**

Housing was not a factor that was observed or assessed at this point in testing. All of the animals included in the cued variant of the MWT were pair-housed with an animal that was exposed to the same prenatal treatment. Because all of the rats were in the same housed condition,
housing is not included as a factor in these analyses. Latency to the goal location decreased for all groups throughout the 6 blocks of training that took place over 2 days of training and testing (see Figure 8, F(5, 120) = 45.23, p < 0.001, $\eta_p^2 = 0.385$). No significant main effects of prenatal treatment (F(1, 24) = 1.74, p = 0.20, $\eta_p^2 = 0.067$) or sex (F(1, 24) = 0.11, p = 0.916, $\eta_p^2 < 0.001$) and no significant interactions (F(1, 24) = 1.60, p = 0.218, $\eta_p^2 = 0.063$) between the 2 factors were observed during the training blocks. All groups performed the same throughout the entire training session, which suggests that all animals were able to sufficiently learn where the goal was located. Additionally, all animals learned to take more direct and efficient routes to the goal location over time, which was apparent by the decreased latency throughout the blocks and days.

**Cued Platform Shift Tests:**

There was a significant effect of shift test (see Figure 9, F(1, 24) = 5.926, p = 0.023, $\eta_p^2 = 0.198$) suggesting that there is a significant difference between how long it took the rats to travel to the place location versus the directional location. No significant main effects of prenatal treatment (F(1, 24) = 1.28, p = 0.27, $\eta_p^2 = 0.05$) or sex (F(1, 24) = 1.08, p = 0.31, $\eta_p^2 = 0.043$) and no significant interactions (F(1, 24) = 2.89, p = 0.102, $\eta_p^2 = 0.107$) between the 2 factors were observed during the cued shift tests. These findings suggest that all of the animals performed in a similar fashion to one another throughout the cued variant of the MWT pool shift manipulation. All of the animals efficiently navigated to the visual platform, which suggests that all of the animals learned where the goal location was and that there were no visual impairments that limited the performance of any animal. Also, the shift tests show that all animals display a preference for the directional location over the place location despite the gestational exposure or sex of the
animal. These data further support that these animals are capable of learning and completing the pool shift manipulation and that they all have a base preference for the directional location when the goal location is visible. It is only when the pool shift task is made more difficult by hiding the goal location that these differences in navigational behavior arise.

**Cued Reversal Training:**

Latency to the reversed goal location was analyzed in a repeated measures ANOVA. The 8 reversal trials were condensed into blocks and analyzed in order to account for the variability in the 4 different drop locations. There were no significant main effects of block (see Figure 10, F(5, 24) = 3.253, p = 0.084, $\eta^2_p = 0.119$), prenatal treatment (F(1, 24) = 1.02, p = 0.322, $\eta^2_p = 0.041$), or sex (F(1, 24) = 0.002, p = 0.966, $\eta^2_p < 0.001$) and no significant interactions (F(1, 24) = 0.0003, p = 0.987, $\eta^2_p < 0.001$) between the 2 factors. All groups performed the same throughout the entire reversal session, which suggests that all animals were able to sufficiently learn where the reversed goal was located. These data also suggest that all of the animals are able to display flexible behavior in the cued variant of the MWT pool shift variant.

**Hippocampal cell firing:**

Data was successfully collected from a total of 16 animals, n=2 for each of the 8 groups. The number of cells in each sex-treatment-housing combination is as follows: Female-Sac-Same (n=28), Female-Sac-Mixed (n=20), Female-PAE-Same (n=88), Female-PAE-Mixed (n=59), Male-Sac-Same (n=15), Male-Sac-Mixed (n=18), Male-PAE-Same (n=78), Male-PAE-Mixed (n=21). There were no significant main effects of sex (F(1, 319) = 2.125, p = 0.146, $\eta^2_p = 0.007$),
prenatal treatment (F(1, 319) = 2.345, p = 0.127, $\eta^2_p = 0.007$), or housing (F(1, 319) = 0.124, p = 0.725, $\eta^2_p < 0.001$) for correlation and no significant main effects of sex (F(1, 319) = 0.029, p = 0.864, $\eta^2_p < 0.001$), prenatal treatment (F(1, 319) = 0.116, p = 0.733, $\eta^2_p < 0.001$), or housing (F(1, 319) = 2.912, p = 0.089, $\eta^2_p = 0.009$) for minimal rotational value (see figure 11). Figure 11 visualizes the comparison between cell firing in the initial location and cell firing in the shift location. Each of the sections (A, B, C, and D) show the rate map and spike firing for cell firing in the initial location, the cell firing after the shift manipulation, and the sum of squared differences between the two rate maps. The sum of squares is calculated between the two cells as one cell is rotated 0-360 degrees and compared to the non-rotated, stationary cell. The rotational comparison allows for the determination of where the cells are minimally and maximally different. The degree where the two cells are least alike is designated as the minimum rotational value and is quantified in order to classify if a cell is bound to the apparatus (A), the distal cues (C), or if they remap or are undetermined (B and D). A cell is determined to be bound to the apparatus and local reference frame if the minimum rotational value was between 0-45° + 315-360°. Apparatus bound cells shift with the apparatus and stay in the same relative location after the shift manipulation. If a cell is bound to the apparatus it would be likely that the spatial navigation behavior displayed by the animal would be a preference for directional navigation. A cell is determined to be bound to the distal cues and distal reference frame where the minimum rotational value fell between 135-225°. Distal cue bound cells do not shift with the apparatus and stay in the absolute location as determined by the distances from different distal cues after the shift manipulation. If a cell is bound to the distal cues it would be likely that the spatial navigation behavior displayed by the animal would be a preference for place navigation. Remapped/undetermined cells were determined to be where the minimum rotational value fell between 45-135° or 225-315°. These cells may explain
an animal not showing a preference for place for directional responding in the MWT shift manipulation because of the cells not being bound to a specific cue. The number of cells for each of the groups were categorized into 90 degree wedges in order to determine how many were bound to the apparatus, the distal cues, or remapped/undetermined (see table 1). These data were also split up into principal cells (4-8 Hz) and interneurons (see table 2). In accordance with the MWT data, mixed housed PAE females should have more cells bound to the distal cues than mixed housed saccharin females. This hypothesis is not supported by the data found in these experiments. Overall, there was a significant three-way sex X treatment X housing interaction (F(1, 319) = 12.4436, p <0.001, η²<sub>p</sub> = 0.038) for the correlation between cells, a significant sex X housing interaction (F(1, 319) = 19.956, p < 0.001, η²<sub>p</sub> = 0.059) for the correlation between cells, and a significant sex X treatment interaction for the correlation between cells (F(1, 319) = 9.883, p = 0.002, η²<sub>p</sub> = 0.030) and for the minimum rotational value (F(1, 319) = 6.015, p = 0.015, η²<sub>p</sub> = 0.019) (see Figure 12).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tx</th>
<th>Housing</th>
<th>Apparatus</th>
<th>Distal</th>
<th>Remap</th>
</tr>
</thead>
<tbody>
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<td>PAE</td>
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<td>38.1% (8)</td>
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<td>75.0% (21)</td>
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<tr>
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<td>61.9% (13)</td>
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<td>3.8% (3)</td>
<td>53.8% (42)</td>
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<tr>
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<td>11.1% (2)</td>
<td>83.3% (15)</td>
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<tr>
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<td>0.0% (0)</td>
<td>66.7% (10)</td>
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Table 1. Categorization of cells that are bound to the apparatus (0-45° + 315-360°), the distal cues (135-225°), or remapped/undetermined (45-135°/225-315°). The percentages and number of cells are both reported.
<table>
<thead>
<tr>
<th>Sex</th>
<th>Tx</th>
<th>Housing</th>
<th>Apparatus</th>
<th>Distal</th>
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<td></td>
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<td></td>
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<td>Inter</td>
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<tr>
<td></td>
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<td>Inter</td>
<td></td>
</tr>
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<td>60%</td>
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</tr>
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<tr>
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<td>7.7%</td>
<td>42%</td>
</tr>
<tr>
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<table>
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<tr>
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<td>42</td>
</tr>
<tr>
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<td>Sac</td>
<td>Mixed</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>F</td>
<td>Sac</td>
<td>Same</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>M</td>
<td>PAE</td>
<td>Mixed</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>M</td>
<td>PAE</td>
<td>Same</td>
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<td>49</td>
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<tr>
<td>M</td>
<td>Sac</td>
<td>Mixed</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>M</td>
<td>Sac</td>
<td>Same</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2. Categorization of principal cells (Principal) and interneurons (Inter) that are bound to the apparatus (0-45° + 315-360°), the distal cues (135-225°), or remapped/undetermined (45-135°/225-315°). The percentages and number of cells are both reported. The percentages are calculated as the number of cells in the specific category divided by the total number of cells (principal or interneuron) in that group. Abbreviations: Female (F), Male (M), PAE (P), Sac (S).
Conclusions:

The current study shows that PAE, sex, and housing all influence the spatial behavior and navigation strategies used by rats in the MWT. Most notably, the mixed-house PAE females showed a preference for place navigation while all other groups showed a preference for directional responding or no preference at all. This research shows how spatial navigation behavior can be influenced by a multitude of variables. The housing manipulation was associated with differential preference for directional or place responding that was sex specific. In addition to this spatial behavior specificity, prenatal alcohol exposure especially altered the behavior of the mixed-housed females differently than all other groups. Previous research has shown that typically-developing rodents have a preference for directional responding, yet this study expands upon previous research by assessing the influence of prenatal alcohol exposure, sex, and housing in a variant of the MWT.

Additionally, this research has shown through a novel analysis that all rats have some cells that bind to the apparatus and some that bind to distal cues and it seems as though the cells that bind to the apparatus are more stable than those that bind to the distal cues. The number of cells that bind to a specific cue or that remap vary depending on the animal’s sex, prenatal treatment, and housing manipulation. When looking at the percent of cells that are bound to the cues all of the rats display a stronger attachment to the apparatus than the distal cues, which is different than what is displayed in the shift manipulation in the MWT. Furthermore, the overall stability of cells throughout the shift manipulation is also significantly affected by sex, prenatal treatment, and housing. This effect is driven by the decreased correlation coefficient in the mixed-housed Sac
males compared to all other groups. The mixed-housed Sac males display a very low correlation of $r = 0.1$ after the shift manipulation while the mixed-housed Sac females display the strongest correlation out of all of the groups $r = 0.44$. Overall, this data suggests that these cells are not particularly stable throughout the shift manipulation.

These data support the hypothesis that spatial navigation behavior is contingent upon sex, housing, and prenatal treatment, but the predictive value of hippocampal cell firing on spatial navigation behavior was not supported. The cells were defined as apparatus bound, distal cue bound, or as a cell that remapped/was undefined. An apparatus bound cell would shift with the apparatus and stay in the same relative location after the shift manipulation and it would be likely that the spatial navigation behavior displayed by the animal would be a preference for directional navigation. Distal cue bound cells do not shift with the apparatus and stay in the absolute location as determined by the distances from different distal cues after the shift manipulation. If a cell is bound to the distal cues it would be likely that the spatial navigation behavior displayed by the animal would be a preference for place navigation. Remapped/undetermined cells may explain an animal not showing a preference for place for directional responding in the MWT shift manipulation because of the cells not being bound to a specific cue. According to the hypothesis and these categorizations of cells mixed housed PAE females should have more cells bound to the distal cues, but this research found that this group had ~40% of their cells bound to the apparatus, ~20% of their cells bound to the distal cues, and ~40% that remapped. Also, the mixed housed saccharin females should have more cells bound to the apparatus, but ~40% of their cells were bound to the apparatus, 30% of the cells were bound to the distal cues, and 30% of the cells remapped or were undefined. Although the mixed housed saccharin females had more cells bound to the apparatus, this finding was not significant. Furthermore, in order to support the hypothesis,
all same housed females should have significantly more cells the remap compared to all other types of cells. The saccharine females showed a trend towards this prediction, but the PAE females do not. Overall, the female data does not support the hypothesis in specific aim 2. The male behavioral data suggests that all of the PAE males and the same housed saccharin males should have significantly more cells that remap compared to all other categories and that the mixed housed saccharin males may show some preferred cell firing that is bound to the apparatus. The data shows that most of the cells in the male animals remap or are undefined. The only group that shows a different trend is the same housed PAE males where about half of the cells are bound to the apparatus and the other half of cells remap. Again, none of these effects are statistically significant and they ultimately do not support the central hypothesis of this dissertation.

The influence of social environment and prenatal alcohol exposure have been studied in various domains, including in the MWT in our laboratory [8, 130-135]. Previous studies have shown that social changes influenced by PAE were ameliorated by social enrichment. Although some may suggest that placing a PAE animal with a saccharin-exposed control animal would be enriching for the PAE animal, the current study shows otherwise. The PAE animals in the mixed-housing condition show a significant difference in spatial responding that does not emulate that of the saccharin-exposed animals. The mixed-housed saccharin-exposed rats display the same behavior found in our previous studies while the mixed-housed PAE rats showed the opposite pattern of responding. These findings suggest that PAE rats are not necessarily enriched solely by the presence of the control rat in the environment. Interestingly, when rats were pair-housed with a rat of the same prenatal treatment, they did not display a discerning difference between place or direction responding in that they took the same amount of time to find the absolute and relative locations. Also, sex differences were observed in PAE rats. Overall, the PAE male rats displayed
similar responding to the absolute and relative locations despite the housing condition. On the other hand, the mixed-housed PAE females display a preference for the absolute location, indicating that they utilize place responding more efficiently than directional responding to the relative location. Again, when the females were placed in the same housing condition, they showed no discernable difference in preference for place or directional responding. When looking at directional responding to the relative location only, the mixed-housed males found the relative location much quicker than the same-housed males while the females showed the opposite in regard to housing. The same-housed females found the relative location much quicker than the mixed-housed females. All mixed-housed rats took significantly longer to find the absolute location compared to the same-housed rats, which is apparent by the latency to the platform location and by the negative preference score. Overall, this data suggests that exposure to alcohol during gestational development and housing manipulations alter spatial behavior and spatial representations in a sex-specific manner.

**General Discussion:**

In summary, FASDs are a global public health problem that requires addition research in order to develop specific treatments for the cognitive dysfunctions associated with prenatal ethanol exposure. FAS represents a small portion of the larger spectrum that is continuously growing. Most children with PAE have neurodevelopmental problems but lack the characteristic facial features of FAS [32]. Prenatal alcohol exposure (PAE) can lead to persistent learning and memory deficits, which are some of the most prevalent symptoms in FASD. Human studies have revealed that children with FAS display impaired spatial learning in the hidden-platform variant of the virtual MWT. Similarly, rodents exposed to high doses of ethanol during gestation
(approximately 200-300 mg/dl) display acquisition deficits in the MWT. Rodents repeatedly exposed to moderate levels of ethanol during gestation (approximately 60-80 mg/dl) are spared from acquisition impairments, but exhibit long-term memory deficits when tested several days after initial training. Complex tasks are more difficult for PAE humans and animals. These cognitive changes may be due to decreased hippocampal volume, decreased hippocampal pyramidal cell counts, and alterations in long-term potentiation.

There are currently no treatments that alleviate the learning and memory deficits in FASD, which may be due to the incomplete understanding of these cognitive alterations. Gaining a comprehensive understanding of the underlying mechanisms involved in FASD related learning and memory deficits are vital for developing potential treatments. PAE effects on hippocampal dependent learning, structures, and LTP have been investigated, hippocampal place-cells have never been identified or assessed in PAE animals. Hippocampal place-cells (HPCs) fire in a spatially-selective manner that is dependent upon the animal’s position in an environment [136, 137]. Place cells were first identified by O’Keefe in 1976 and have since become well defined in typically developing systems [144]. In the MWT, directional responding involves control of behavior by a distal cue frame of reference for orientation and a local frame of reference (the pool apparatus) for localization of a goal, whereas place navigation involves control of spatial behavior by the distal reference frame to guide spatial localization [13]. Local apparatus and distal cue frames of reference also differentially influence neural representations of space realized in the activity of HPCs, which preferentially represent locations in local reference frames with a smaller population of HPCs representing locations defined by distal reference frames [14-19]. This approach may provide significant information about the underlying mechanisms of FASD-related
alterations in spatial cognition, learning, and memory, which may be vital for the development of therapeutic treatments.

Previous studies have characterized the influence of PAE on spatial navigation and behavioral flexibility through various behavioral paradigms including the MWT. The current study examined the behavioral consequences of PAE on directional and place navigation through the use of the MWT and the stability of hippocampal place fields in a dry land variant of the shift paradigm. The animals were tested in a 2-day hidden platform protocol, a 2-day cued platform protocol, and in vivo electrophysiology recordings were collected to assess HPCs in the shift manipulation. There were no significant differences between PAE treatment or sexes in the hidden or cued training trials. In the hidden variant there was a significant interaction in the females between preference for the absolute or relative location and PAE. The SAC female rats displayed a preference for the relative platform location, which is consistent with previous findings, while PAE female rats displayed a preference for the absolute location. The behavioral outcomes do not exactly match those of the in-vivo electrophysiology research. Freely, moving in-vivo electrophysiology measures were acquired in a dry land version of the shift manipulation. These recordings showed a significant sex X treatment X housing interaction which was driven by a decreased correlation in the mixed-housed Sac males. Overall, the mixed-housed PAE females show differential spatial navigation behavior in the MWT and the mixed-housed Sac males show a decreased hippocampal cell correlation through the dry land version of the shift manipulation. These outcomes suggest that animals may have distinct search patterns that are sex and housing specific and can be influenced by PAE, but these behavioral changes are not necessarily due to alterations in hippocampal firing. There are few effective treatments for the behavioral and
cognitive consequences of gestational exposure to prenatal alcohol exposure. Further research needs to be conducted in order to fully understand the mechanisms behind PAE.

The reasons why PAE and control animals and why females and males respond differently depending on the social environment are not understood. One proposed hypothesis was that these changes could be due to an alteration of frames of references in PAE mixed-housed females that are not present within the other groups. In the MWT, directional responding involves control of behavior by a distal cue frame of reference for orientation and a local frame of reference (the pool apparatus) for the localization of a goal, whereas place navigation involves control of spatial behavior by the distal reference frame to guide spatial localization [13]. Hippocampal place-cells (HPCs) fire in a spatially-selective manner that is dependent upon the animal’s position in an environment[136,137]. Local apparatus and distal cue frames of reference also differentially influence neural representations of space realized in the activity of hippocampal place-cells (HPCs; neurons that increase firing when an animal occupies a particular location), which preferentially represent locations in local reference frames with a smaller population of HPCs representing locations defined by distal reference frames[14]. One possible explanation for the behavioral differences is that an alteration in these reference frames is associated with a stronger population of place cells associated with the place location in the mixed-housed PAE females that are not as strong or present in all other groups. Even though there is a clear difference in behavioral performance this does not necessarily translate to the in-vivo data. This research suggests that these frames of references are not specifically disrupted in the mixed-housed PAE females as was hypothesized. There were significant interactions between sex, treatment, and housing, but it was not specific to the mixed-housed PAE females. This significant interaction is driven by the weakest
correlation in the mixed-housed Sac males, but these differences could be due to decreased number of cells identified in that group.

The research conducted here suggests that hippocampal cell firing may contribute to the output of spatial navigation behavior, but may not be sufficient or the only input that influences the behavioral outcomes. Previous research has also suggested that hippocampal cell firing is not sufficient for the determination of spatial behavior outcomes. McNaughton et al. (1989) destroyed over 75% of the dentate gyri granule cell input into the CA1 and CA3 fields of the hippocampus, which lead to spatial learning deficits while keeping place cell fields intact [145]. This is particularly interesting in regard to the current study because it may explain why mixed housed PAE female rats have altered spatial navigation preferences, but do not show an alteration in hippocampal cell firing. The current study did not analyze neuronal differences in the dentate gyrus or in other inputs to the CA1 or CA3, such as the entorhinal layers II and III. There may be decreased input or a decreased number of cells firing from the dentate gyrus or the entorhinal cortex to the CA1 that results in spatial behavior alterations while sparing place fields in the CA1. Additionally, the current study only recorded hippocampal cells in the right CA1, while McNaughton et al. (1989) conducted their study bilaterally. Future research should include recordings from both hemispheres in order to obtain a more sufficient understanding of the effects of PAE, sex, and housing on place fields and behavioral outcomes.

Input to the hippocampus and other circuits may also contribute to the output of spatial navigation behavior in addition to hippocampal place cells. Stackman et al. (2012) reported a preference for directional responding in the pool shift version of the MWT, but directional responding to the relative location in the pool could be manipulated by disorienting the mice and by selective inactivation of the anterior thalamic nuclei [146]. Rotating the distal cues outside of
the maze guided responding in the same direction as the cues were rotated and disorientation through gently rotating the mouse in an enclosed box shifted the preference for directional responding to having no preference for navigating to the place or directional locations. These results suggest that spatial navigation behavior utilizes information from both internal and external cue inputs. The rodents utilized in this research could be utilizing internal and/or external cues differently in the MWT that leads to directional behavioral responding that may not be utilized in the dry-land open field leading to different neural representations being recorded. Additionally, multiple circuits may be involved in altering spatial navigation behavior that was not studied in the current research. For example, Stackman et al. (2012) found that selective inactivation of the head direction cell circuitry in the anterior thalamic nuclei through bilateral infusions of muscimol lead to a shift to a preference for place responding from directional responding [146]. This shift in spatial navigation behavior was only present after inactivation of the anterior thalamic nuclei and did not occur when the CA1 region of the hippocampus was inactivated. The recordings in this study were only conducted in the CA1 region of the hippocampus. The stark difference in place versus directional responding found in the Stackman et al. study strongly suggests that the alterations in spatial navigation behavior may be more heavily influenced by alterations in the anterior thalamic nuclei and may not be sufficiently explained by changes in the CA1.

Additionally, Sutherland et al. (1997) observed long lasting alterations to hippocampal plasticity via induction of long-term potentiation (LTP) in adult rats that were prenatally exposed to ethanol [147]. These animals were exposed to a 5% ethanol liquid diet during gestational development, which resulted in a peak maternal blood ethanol concentration of 83 mg/dl, which is comparable to the research conducted in this dissertation. The PAE rats showed deficits in field EPSPs after exposure to high-frequency stimulation. This research is particularly interesting in the
scope of this dissertation because LTP plays a significant role in place fields and are vital in place field stability over time [148]. If LTP is selectively diminished in rats exposed to ethanol during gestational development, then the downstream effects could be altered place fields or unstable place fields over time. This type of research has not been conducted in male and female PAE rats who have experienced housing manipulations, but LTP deficits could play a part in the research conducted here. Furthermore, LTP is contingent upon functioning NMDA receptors, which multiple studies have found to be decreased after prenatal ethanol exposure [149-151]. The decreased expression of NMDA receptors in the hippocampus may lead to LTP deficits which could ultimately result in less stable place fields or alterations in place cell characteristics. These circuits and systems need to be addressed in future studies.

**Limitations and Future Studies:**

For over a decade, rat offspring from this model of moderate prenatal alcohol exposure have been employed in spatial navigation studies within multiple laboratories [122, 132]. Although this moderate exposure paradigm is informative and translatable to the human condition, additional experiments are needed in order to assess the effect of higher alcohol exposure and alcohol exposure at different time points in development on spatial navigation behavior. Previous work has shown that exposure to high levels of ethanol during gestational development leads to impaired spatial memory and behavior in the MWT, but it is still unknown how these high exposure levels could alter behavior in the shift paradigm utilized in this study. Utilizing this variant of the MWT focuses on *how* a spatial task is completed and not just *if* spatial learning can take place, which is sometimes lacking in studies. Even more so, future research could focus more on low-moderate levels of ethanol exposure since the majority of women who drink (knowingly or unknowingly)
while they are pregnant do not binge drink every day and the majority of FASD cases fall in the less severe range of the spectrum [138]. Current research suggests that the behavioral consequences of moderate levels of ethanol \textit{in utero} tend to be undetectable under basic behavioral assessments owing to the subtle nature of these effects, however, understanding these effects and their neurobiological underpinnings is critically important for understanding mechanisms and pursuing treatments for fetal alcohol spectrum disorders (FASD). Moderate PAE consistently spares spatial learning in the MWT, however, whether moderate PAE alters spatial strategies has not been determined. Children with FASD display a myriad of cognitive deficits that are complex in nature and the lack of initial learning deficits does not negate the possibility that moderate PAE can alter cognitive abilities and influence behavioral strategies. Several studies have dissociated directional navigation, responding towards a certain direction in the environment based on visual information, and place navigation, responding to an absolute location in the environment based on visual information [9]. Assessments to differentiate spatial navigation strategies have been used in a multitude of human and rodent studies [19, 139-142]. Typically developing humans and rodents prefer to use directional responding when solving dry mazes and multiple MWT variants [12, 13, 143], but these tasks have never been utilized in the assessment of PAE effects. Utilizing complex variants of the MWT in rodent PAE models is important for understanding the effects of moderate prenatal ethanol on how rats solve spatial tasks, instead of solely whether moderate PAE rats can solve spatial tasks, which this study focuses on. Future animal studies could also focus on more common doses, frequency, and timing found in human maternal consumption since most of the population affected by PAE has neurodevelopmental problems without having the entire profile of characteristic involved in FAS. For example, no studies address the effects of intermittent binge drinking during the first two trimesters of gestation. This is particularly relevant because women
may partake in binge-drinking on the weekends before knowing that they are pregnant. This type of animal model could be particularly beneficial and could easily be utilized with the methodology described in this paper.

There are contradicting sex differences in many PAE studies, including this one, suggests that additional research is needed to parse out sex-dependent PAE alterations. This study builds upon previous work by including prenatal alcohol exposure, sex, and housing manipulations and by using a more difficult variant of the pool shift task where the goal location is hidden, but these data is still unclear as to why these changes are occurring. Including sex and housing are particularly important to include in future studies because there many be different baselines of behavior displayed in these populations that may be standard, but could be falsely interpreted as deficits or, worse, may be interpreted as false support for future treatments. These factors must be taken into consideration for all future work as the interaction of these three factors may influence behaviors and outcomes more than previously thought.

The work conducted in this study included a total of 87 animals split unevenly throughout the 8 groups in the MWT experiments and 16 animals in the in vivo electrophysiology experiment. An increase in number of animals in order for there to be an equal number of subjects in each group may further explain or enhance the group differences in this study. Although there was an even number of animals in the electrophysiology experiments, there was a vast difference in the number of cells and comparisons completed. Redoing these experiments with an increase in numbers, both animal and cell, could help parse out what is really occurring in this phenomenon. More importantly, this research was conducted in multiple sets of animals. It would be ideal to conduct the MWT and electrophysiology assessments in the same animals so that all of the data can be directly correlated to one another.
This study was also conducted during adulthood (>5 months) only. Observing the development of spatial behavior and hippocampal cell firing throughout multiple time frames, such as adolescence and early adulthood, may provide a more thorough explanation as to how social environment interacts with PAE and sex. Understanding these factors throughout multiple time points in maturation and aging may be important for future studies and may provide insight as to why behavior is present in one time point and not another. These future studies could also help with the development of age-appropriate interventions that would not be possible or misguided based on this information from this one time point.

This research includes housing manipulations as a factor by pair-housing rats with a same sex partner of a different or the same gestational exposure. The animals stay its respective housing manipulation prior to and throughout the entirety of the MWT sessions, but the animals that underwent surgery are housed individually post implantation. This difference in housing may alter any results shown here and make it very difficult to correlate the MWT and in vivo recordings to one another. Standard post-surgery care dictates that rats need to be individually housed to promote proper healing and to decrease the chance of the Microdrive being dislodged. Future research could rehouse the rats with its original housing partner in order to make sure that the housing manipulation is as effective as possible. Additionally, housing manipulations could be further investigated by group housing the rats in mixed and same prenatal treatment groups.

Future research may also want to focus on including more goal-oriented versions of the dry land shift manipulation. In this research was the first to look at the effects of PAE, sex, and housing in vivo recordings in freely moving rats in the shift manipulation. To that point, the rats were free to explore (or not explore) as much as they want. The presence of a sugary treat provided some incentive to explore the entire apparatus, but this variant did not include a goal location equivalent
to the platform location in the MWT variant. Further research may want to include a salient goal that may motivate behavior in a similar way that an escape platform does. One possible way of doing this is through food deprivation. Many studies involving in vivo electrophysiology in awake, freely moving animals limit the amount of food that animals have access to, which makes the food during the recording session more salient.

Additionally, further analyses of this data need to be conducted. Research conducted in Benjamin Clark’s laboratory as examined how PAE disrupts peak firing rates, within-session stability of place fields, and theta modulation in male rats in a linear track. Combining the analyses of place field characteristics with the shift manipulation and including sex and housing as factors could be very beneficial for the field. The rotational analyses could also be further utilized in future studies and could be very informative when looking at different manipulations, such as cue directed behavior in this population. Furthermore, these studies were only conducted on the CA1 region of the hippocampus. It is possible that the input into these HPCs could be altered, which leads to a disruption in spatial navigation behavior. It could be very informative if recordings could be taken from other areas of input, such as the perforant pathway from the entorhinal cortex, the Schaffer collateral pathway in the CA3, or the dentate gyrus. These additional regions of the hippocampus would be of particular interest because previous research has shown that PAE alters hippocampal cells and NMDARs, which are vital for long-term potentiation and spatial learning and memory. The rats in this research have displayed sufficient learning in the MWT, but there are still alterations in how those spatial tasks are completed. Assessing other mechanisms, such as long-term potentiation, throughout all of these groups may better explain the modified spatial behavior and could also explain alterations in the stability of the hippocampal place fields in this study. The behavior in these rats may be a result of a host of systemic alterations such as entorhinal
input damage, impaired directional coding, or impaired directional systems. In conjunction with possible HPC differences, other cell systems could be influencing spatial navigation behavior such as head direction cell, grid cells, or border cells, as they all play a role in spatial learning.

The behavioral consequences of moderate levels of ethanol in utero tend to be undetectable under basic behavioral assessments owing to the subtle nature of these effects. However, understanding these effects and their neurobiological underpinnings is critically important for understanding mechanisms and pursuing treatments for fetal alcohol spectrum disorders (FASD). This study has built upon several other studies which have dissociated directional navigation and place navigation. Moderate PAE consistently spares the acquisition of spatial learning in the MWT and this study has established that moderate PAE alters spatial strategy preferences. However, it has yet to be determined why this preference has shifted, especially in female rodents prenatally exposed to alcohol and brought up in a mixed prenatal treatment environment. Future research should be aimed towards understanding alterations in the use of spatial strategies (directional vs. place responding) in a rodent model of PAE and the underlying neural mechanisms at the systems level. Frames of reference are a central conceptual aspect of this work. In the MWT, directional responding involves control of behavior by a distal cue frame of reference for orientation and a local frame of reference (the pool apparatus) for localization of a goal, whereas place navigation involves control of spatial behavior by the distal reference frame to guide spatial localization [13]. Local apparatus and distal cue frames of reference also differentially influence neural representations of space realized in the activity of hippocampal place-cells (HPCs; neurons that increase firing when an animal occupies a particular location), which preferentially represent locations in local reference frames with a smaller population of HPCs representing locations defined by distal reference frames[14]. Future studies can assess how moderate PAE induces
alterations in spatial navigation strategies, which are related to alterations in neural representations of spatial reference frames. These studies would help us understand the underlying mechanisms that lead to alterations in spatial behavior, which are depend upon in utero exposure, sex, and housing conditions and may lead to better therapeutic targets for those exposed to alcohol during gestational development.
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Figure 1. Pool shift to assess spatial navigation behavior. Rats were trained to navigate to an escape platform in a circular pool. All rats were trained to the place location (P) when the pool was located in the training location. For each navigation strategy test the pool was shifted 0.75m to the shift location and the platform was moved relatively with the pool to assess directional responding (D) or placed in the absolute location in the room to assess place responding (P).
Figure 2. Hidden platform training - Latency to reach the goal location for female (A) and male (B) rats throughout 6 blocks of training across 2 days. There is a significant effect of block, but no significant interactions or main effects of prenatal treatment, sex, or housing. The training was split up by sex for visual purposes only.
Figure 3. Hidden platform shift tests - Latency to reach the goal locations (directional or place) for female (A) and male (B) rats after the apparatus was shifted the distance of the radius of the pool. The mixed-housed rats and the same-housed rats showed a significant main effect of sex of $p < 0.05$. 

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Figure 4. Hidden platform shift tests - Latency to the goal locations. There is a significant shift test X prenatal treatment X housing multi-variant interaction. Asterisk (*) indicates a significant interaction of $p < 0.05$. 
Figure 5. Latency to the goal locations. PAE animals displayed a significant housing X sex interaction. Asterisk (*) indicates a significant interaction of p < 0.05.
Figure 6. Hidden shift test – A significant housing X sex interaction was observed for the latency to the directional location. Asterisk (*) indicates a significant interaction of p < 0.05.
Figure 7. Quantification a preference for the directional or place location = (latency to directional location – latency to place location)/(latency to directional location + latency to place location). A negative value represented a preference for the place location and a positive value represented a preference for the directional location. There was a main effect of housing for this preference score. Asterisk (*) indicates a significant effect of p < 0.05.
Figure 8. Cued platform training - Latency to reach the goal location for female and male rats throughout 6 blocks of training across 2 days. There is a significant effect of block, but no significant interactions or main effects of prenatal treatment or sex. These data show that all of the animals learned where the visible goal location sufficiently and equally.
Figure 9. Cued platform shift tests - Latency to reach the goal locations (directional or place) for female and male rats after the apparatus was shifted the distance of the radius of the pool. Asterisk (*) indicates a significant effect of $p < 0.05$. 
Figure 10. Cued reversal trials - Latency to reach the goal location for female and male rats throughout 2 blocks of reversal trials. There are no significant main effects of trial, prenatal treatment, or sex.
Figure 11. Hippocampal cell firing – comparison between the initial location firing and shift location firing. Each of the sections (A, B, C, and D) show the rate map and spike firing for cell firing in the initial on the top, the shift cell firing on the bottom, and the sum of squared differences between the two cells on the right. The sum of squares are calculated between the two cells as one cell is rotated 0-360 degrees and compared to the non-rotated, stationary cell. The x axis on all of the SS difference maps starts at 0 and goes up to 360 in order to determine where the cells are most and least alike. The minimum rotational value is quantified in order to classify if a cell is bound to the apparatus (A), the distal cues (C), or if they remap or are undetermined (B and D) A. Apparatus bound cell where the minimum rotational value was between 0-45° + 315-360°. B. Remapped/undetermined cell where the minimum rotational value fell between 45-135°. C. Distal cue bound cell where the minimum rotational value fell between 135-225°. D. Remapped/undetermined cell where the minimum rotational value well between 225-315°.
Figure 12. Correlation coefficients to compare the cells prior and post shift. There was a significant three-way sex X treatment X housing interaction, a significant sex X housing interaction, and a significant sex X treatment interaction for the correlation coefficients. Asterisk (*) indicates a significant interaction of $p < 0.05$. 