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An Analysis Of Virtual Place Learning/navigation In Children And Young Adults Prenatally Exposed To Alcohol

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AN ANALYSIS OF VIRTUAL PLACE LEARNING/NAVIGATION IN CHILDREN AND YOUNG ADULTS PRENATALLY EXPOSED TO ALCOHOL

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CHAPTER 1 INTRODUCTION

1.1 Background

Fetal alcohol spectrum disorders (FASD) is the umbrella term used to describe the broad range of adverse outcomes associated with prenatal alcohol exposure (Hoyme et al., 2005). Fetal alcohol syndrome (FAS), the most severe form of FASD, is characterized by microcephaly, pre- and or postnatal growth retardation, and a distinctive set of craniofacial dysmorphic features, including short palpebral fissures, thin upper lip, and a smooth philtrum. Partial FAS (PFAS) is defined as the presence of two of the three key alcohol-related facial features with microcephaly, growth retardation, or cognitive and/or behavioral dysfunction. The most prevalent form of FASD, alcohol-related neurodevelopmental disorder (ARND) describes alcohol-exposed individuals who lack the craniofacial dysmorphology but exhibit mild to moderate neurobehavioral deficits (Stratton, Howe, & Battaglia, 1996) and is the most difficult to identify. Although many children with FAS and PFAS are intellectually disabled and often exhibit behavioral problems, some perform in the low average to average IQ range (Streissguth et al., 1991). In ARND, subtle effects on IQ and behavioral dysfunction have been detected (Mattson, Riley, Gramling, Delis, & Jones, 1997; Streissguth, Barr, & Sampson, 1990), however these effects become particularly apparent in certain subgroups, such as children of mothers who are older and or have a history of alcohol abuse (Chiodo et al, 2010; Jacobson, Jacobson, Sokol, Chiodo, & Corobana, 2004) or those born to mothers who lack a particular alcohol metabolism allele (Dodge, Jacobson, & Jacobson, 2014; S. Jacobson et al., 2006; McCarver, Thomasson, Martier, Sokol, & Li, 1997).

Many neurocognitive and behavioral deficits associated with prenatal alcohol exposure have been identified including verbal learning (Coles, Lynch, Kable, Johnson, & Goldstein, 2010; Lewis et al., 2015; Mattson, Riley, Delis, Stern, & Jones, 1996), number processing (Goldschmidt, Richardson, Stoffer, Geva, & Day, 1996; Jacobson, Dodge, Burden, Klorman, & Jacobson, 2011; Kopera-Frye, Dehaene, Streissguth, 1996), attention and executive function (Burden, Jacobson, Sokol, & Jacobson, 2005; Coles et al., 1997; Kodituwakku, Handmaker, Cutler, Weathersby, &

Handmaker, 1995), slower cognitive processing speed (Burden, Jacobson, & Jacobson, 2005; Coles, Platzman, Lynch, & Freides, 2002; Jacobson, Jacobson, Sokol, & Ager, 1993; Jacobson, Jacobson, & Sokol, 1994), classical eyeblink conditioning (Coffin, Baroody, Schneider, & O'Neill, 2005; Jacobson et al., 2008; S. Jacobson et al., 2011), and non-verbal learning (Aragon et al., 2008; Uecker & Nadel, 1998).

Consistent with the broad range of neurocognitive and behavioral effects, there is evidence of structural brain abnormalities, particularly in the cerebellum, parietal lobes, corpus callosum, and caudate nucleus (Archibald et al., 2001; Lebel, Roussotte, & Sowell, 2011; Sowell et al., 2001; Sowell et al., 2002), as well as compromised white matter integrity (Fryer et al., 2009; Li, Coles, Lynch, & Hu, 2009; Ma et al., 2005; Sowell et al., 2008; Spottiswoode et al., 2011; Treit et al., 2013; Wozniak et al., 2009). Additionally, several functional neuroimaging studies have provided evidence of prenatal alcohol-related neural dysfunction in the domains of verbal learning (O'Hare et al., 2009; Sowell et al., 2007), working memory (Diwadkar et al., 2013; Spadoni et al., 2009), and number processing (Meintjes et al., 2010; Santhanam, Li, Hu, Lynch, & Coles, 2009).

The identification of fetal-alcohol related disorders is often difficult because many affected children do not present with the distinctive facial characteristics and growth retardation, and information regarding prenatal exposure is often lacking. Many standard neuropsychological and behavioral tests may be sensitive to the effects of FASDs, however, they lack the specificity to distinguish FASDs from other neurobehavioral disorders that have similar behavioral phenotypes, such as attention deficit/hyperactivity disorder (ADHD), because they assess complex cognitive and behavioral processes that extend across multiple neurological and neuropsychological domains. The use of assessments that focus on more specific neurocognitive and behavioral processes, such as eyeblink classical conditioning (Coffin et al., 2005; Jacobson et al., 2008; S. Jacobson et al, 2011), oculomotor control (Green, Munoz, Nikkel, & Reynolds, 2007; Green et al., 2009), and number processing (J. Jacobson et al., 2011) have shown to be promising.

A consistent effect of prenatal alcohol exposure on a test of hippocampal-dependent spatial learning, the Morris Water Maze, has been observed in the rodent literature (e.g., Berman & Hannigan, 2000). In this widely-used task, rodents are required to learn the location of a hidden platform to escape from a pool of opaque water. The rodents are able to learn the location of the hidden platform based on the arrangement of distal environmental cues that are present in the room. A human version of the Morris Water Maze has been created with the use of 3-dimensional computer-generated environments. To date, only one study has been published that examined the effects of prenatal alcohol exposure on the virtual water maze (VWM) in humans (Hamilton, Kodituwakku, Sutherland, & Savage, 2003). This study found a dissociation in which the FAS group was impaired in the ability to find the hidden platform based on distal environmental cues (place learning) but did not differ from controls in the ability to navigate to the platform when it was visible (cued navigation). These findings of a behavioral dissociation between place learning and cued navigation in adolescents with FAS are consistent with data from laboratory animals with hippocampal damage and rodent models with prenatal ethanol exposure.

1.2 Statement of Problem

Findings from the Hamilton et al. (2003) study show that virtual place learning has the potential to be a valuable marker to detect the effects of prenatal alcohol exposure and identify alcohol-affected children. First, rodent models examining prenatal alcohol effects on the Morris Water Maze have found specific effects of prenatal alcohol exposure on place learning and not cued navigation (Blanchard et al., 1987; Goodlett et al., 1987). The Hamilton et al. study replicated these specific findings on a human analog of the MWM, thus showing the specificity of fetal alcohol-related place learning deficits translates to human studies. Second, place learning has been shown to be dependent upon specific neurocircuitry in the hippocampus in both rodents (Morris et al., 1982) and humans (Astur et al., 2002). Lastly, rodent studies have reliably shown hippocampal damage as a result of prenatal alcohol exposure (Bonthius & West, 1990). Despite these findings, no other studies in humans have been published examining the effects of FASD

on virtual place learning. The findings from the Hamilton et al. study raise many important questions about the etiology of their observed deficits, and a number of confounding factors exist due to the limitations of their sample. The purpose of this study is to provide a systematic analysis of the effects of prenatal alcohol exposure on virtual navigation in multiple cohorts while controlling for factors that are known to affect virtual and real-world navigation ability and to examine potential mediators of these effects.

1.3 Literature Review

1.3.1 The Morris Water Maze

Over 30 years ago, Richard G. Morris first described his water maze as a tool to investigate spatial learning in rodents (Morris, 1981). Most commonly known as the Morris Water Maze (MWM), it is one of the most frequently used rodent laboratory paradigms in behavioral neuroscience. It consists of a large circular pool that is filled with opaque water, which hides a small escape platform just below the surface. The platform is placed in the middle of one of four imaginary quadrants. Rodents are placed in the pool at random entry points along the circumference and through multiple trials learn the location of this platform to escape the pool. Normal rats quickly learn to swim directly toward the platform from any starting position by learning the spatial position of the hidden platform relative to distal cues present in the room.

The most common procedure used for the MWM consists of three phases: acquisition, probe, and cued navigation. The acquisition phase includes a series of learning trials in which the platform is hidden and the rodent is placed at random entry points along the circumference of the pool. During early acquisition trials, the rat swims across the pool in an exploratory manner until it finds the escape platform. Across acquisition trials, the rat's path to the escape platform becomes more direct as it learns the location of the platform. The process of learning the location of the escape platform is often referred to as *place learning*. Following the acquisition phase, probe trials are performed in which the rat is again placed at a random entry point along the circumference, but the platform has been removed from the pool. During the *probe* trial, rodents

that learned the location of the platform during acquisition trials will spend a greater proportion of time swimming in the quadrant where the platform was located. After a set amount of time has elapsed, the rat is then removed from the pool. Cued navigation trials are performed in which the escape platform is made visible during testing. Performance is typically assessed by measuring the rodent's latency to find the platform, the length of the path traveled to find the platform, as well as the proportion of time spent swimming in the platform quadrant on the probe trial when the platform is removed.

There are three strategies an animal can use to reach the escape platform during swimming trials (Brandeis, Brandys, & Yehuda, 1989). The *praxis* strategy involves the use of a learned sequence of movements to find the platform. This strategy is only efficient if the starting location and platform maintain the same positions relative to each other. The *taxis* strategy is a method in which local, proximal cues that are often visual are used to find the platform. The effectiveness of this strategy is dependent on whether the local cues are visible from the starting point. The third strategy is the *mapping* strategy in which the rodent uses the spatial configuration of distal environmental cues to learn the relative location of the platform in relation to these environmental cues. As long as the platform and distal environmental cues remain stationary, the rodent can use this strategy to navigate to the escape platform from any starting point. Rodents likely use a combination of these navigational strategies, but in standard MWM procedures where the escape platform remains stationary and starting points are random, the mapping strategy appears to be the most useful. Additionally, the effectiveness of the mapping strategy is dependent upon allowing the rat adequate time to view the environment from the location of the escape platform (Sutherland & Linggard, 1982). Rats allowed continuous rearing on the escape platform at the end of each acquisition trial perform better than rats not allowed this experience (Sutherland & Dyck, 1984; Sutherland & Linggard, 1982). This experience of viewing the room from the escape platform allows the rodent to process the relative location of the escape platform in relation to the distal environment cues. The MWM is a test of associative learning. More

specifically, the MWM measures the ability of the rodent to associate environmental cues with the location of an invisible goal. These learned associations encode geometric relations between distal environmental cues and the escape platform to form a cognitive map of the environment (Pearce, Robert, & Good, 1998). Altering the location of these cues or removing them after acquisition disrupts the ability of the rodent to find the escape platform.

1.3.2 Neural correlates of MWM performance

The integral role of the hippocampus in MWM performance was first demonstrated by Morris, Garrud, Rawlins, and O'Keefe (1982). This study found that rats with total hippocampal lesions had a profound impairment in the ability to learn the location of the hidden escape platform. The hippocampal-lesioned group did show some improvement over 8 days of 28 trials of training, but they never reached the levels of the control group. Furthermore, rats with intact hippocampi but with lesions to the neocortex did not show impairment on the MWM. Degree of MWM impairment is found to be related to the volume of hippocampal lesions, and dorsal hippocampal lesions produce more profound effects than lesions to the ventral regions (Moser, Moser, & Anderson, 1993; Moser, Moser, Forrest, Andersen, & Morris, 1995). Pearce and colleagues (1998) found that rats with hippocampal lesions are able to locate a hidden escape platform using heading vectors that specify direction and distance of the goal from a single local landmark that was located within the pool. These same rats, however, were unable to find the escape platform under the more traditional MWM setting consisting of only distal environmental cues, suggesting that the role of the hippocampus in navigation is to facilitate the encoding of geometric relations between the goal and two or more distal landmarks. Pharmacological inactivation of the hippocampus during the acquisition phase produced rats that swam around aimlessly during the probe trial (Riedel et al., 1999), demonstrating the importance of hippocampal function for the encoding of spatial information. However, when rats that were allowed normal hippocampal function during acquisition but underwent hippocampal inactivation only during probe trial testing displayed the same focused searching techniques as controls, but nonetheless searched

inappropriate locations. This study demonstrated that in addition to spatial encoding, the hippocampus is also necessary for the retrieval of location information but is not necessary to retrieve the swimming strategy. To summarize, hippocampal integrity is necessary for successful performance on the MWM. The hippocampus functions to allow the encoding and retrieval of the geometric associations formed between the hidden escape platform and distal environmental cues during acquisition.

The encoding and retrieval processes of the hippocampus during MWM performance are dependent upon NMDA receptor function and long-term potentiation. Morris, Anderson, Lynch, and Baudry (1986) were the first to show that blockade of NMDA receptor impairs hidden-platform performance by administering AP5, a NMDA receptor antagonist into the cerebral ventricles. Further examination showed that intracerebroventricular administration of AP5 impaired MWM performance by blocking long-term potentiation in the hippocampus. More support that NMDA-dependent plasticity in the hippocampus is crucial for MWM performance comes from mice lacking the NR1 subunit of the NMDA receptor in the CA1 area of the hippocampus. These CA1-NR1 knockout mice are severely impaired in their ability to learn the location of the hidden platform but show no deficit during cued-navigation when the platform is visible (Tsien, Huerta, & Tonegawa, 1996). Place cell recordings in these CA1-NR1 knockouts during traversal of a linear track revealed that the place cell fields were larger and less structured and that the temporal coordination of the firing of place cells with overlapping place fields was reduced (McHugh, Blum, Tsien, & Wilson, 1996), suggesting a possible mechanism for impaired MWM performance in these knockout mice. A more recent study has provided evidence that mastery of the MWM involves the use of parallel neural networks involving the CA1 and CA3 regions of the hippocampus along with the parietal cortex (Conejo, Gonzalez-Pardo, Gonzalez-Lima, & Arias, 2010).

Other brain regions have also been found to mediate MWM performance, but their role is less clear. Lesions to the striatum impair the ability to learn the location of the hidden escape

platform (Block, Kunkel, & Schwarz, 1993; Whishaw, Mittleman, Bunch, & Dunnett, 1987), as well as lesions to the nucleus basalis (Waite, Chen, Wardlow, & Thal, 1994), which provides a major source of cholinergic innervation to the cortex and hippocampus. Damage to the cerebellum has also been shown to impair MWM learning (Lalonde, 1994). Rats with one of the cerebellar lobes removed were impaired in their ability to learn the location of the hidden platform but also displayed abnormal exploratory behaviors during testing suggesting that motoric/procedural aspects of navigation may have been affected (Petrosini, Molinari, & Dell'Anna, 1996). Specific cortical regions also appear to be involved in MWM performance. Lesions to the prefrontal cortex impair MWM learning, while parietal lesions do not (Mogensen, Pedersen, Holm, & Bang, 1995). Rats with prefrontal cortical lesions were able to use the distal cues to find the escape platform, but they had a poorer execution of the required course of movements as indicated by longer path lengths (Granon & Poucet, 1995; Mogensen et al., 1995).

1.3.3 Sexual dimorphism on the Morris Water Maze

Sexual dimorphism on MWM performance has been a consistent finding in rodents (Brandeis et al., 1989; Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996; Roof & Stein, 1999), where males perform better than females. These sex differences appear to be related to sex hormones and how they influence the development of the hippocampus.

Testosterone appears to masculinize the hippocampus during development and enhances MWM performance, but experimentally altering testosterone levels during development produces different effects in male and female rats. In adult rats that were administered testosterone during the neonatal period, the effects of testosterone treatment on MWM performance differed by sex (Roof, 1993). Control males performed better than control females, as expected. Neonatal testosterone treatment, however, enhanced MWM performance in the female rats but impaired performance in male rats. Female rats treated with neonatal testosterone also had larger granule cell layers of the dentate gyrus than non-treated females and this increase in granule cell layer size correlated with enhanced MWM performance in females (Roof & Havens, 1992). The

sexually dimorphic effects of neonatal testosterone on water maze performance and hippocampal morphology suggest that there is an optimal level at which testosterone enhances hippocampally-mediated spatial memory and navigation functions during development and that deviations from this level disrupt proper development (Peterson, 1976). In females, the neonatal testosterone treatment described above raised their naturally-occurring lower levels of testosterone, thereby facilitating the development of a more masculine-like hippocampus that enhanced spatial memory function. In the male rats, the increased levels of neonatal testosterone apparently brought them out of the optimal range and disrupted the normal development of male spatial memory functions. Both activation and blockade of androgen receptors in the hippocampus (Naghdi, Nafisy, & Majlessi, 2001) and amygdala (Naghdi, Oryan, & Etemadi, 2003) in adult rats appear to impair water maze performance, suggesting that even acute alterations in testosterone can affect spatial navigation and memory.

Notable effects of female sex hormones have also been found to affect performance on the MWM. Female meadow voles that have naturally circulating high levels of estrogen perform worse than females with lower levels of estrogen (Galea, Kavaliers, Ossenkopp, & Hampson, 1995), and ovariectomized females have enhanced performance on the water maze as compared to females with intact ovaries (Daniel, Roberts, & Dohanich, 1999). During proestrus, when circulating levels of estrogen and progesterone are higher, females perform worse on the MWM than when in estrus (Warren & Juraska, 1997). In ovariectomized females rats, the administration of both estrogen and progesterone together impair MWM performance (Chesler & Juraska, 2000), and in ovariectomized meadow voles, MWM performance was impaired by estrogen administration (Galea, Lee, Kostaras, Sidhu, & Barr, 2002).

The findings from these studies suggest that a reduction of female hormones is associated with better performance on the MWM. However, the mechanism by which this occurs is not clear. Estrogen has been shown to enhance LTP in the hippocampus (Bredemann & McMahon, 2014; Spencer-Segal et al., 2012) and reduce the suppression of hippocampal synaptic activity (Huang

& Woolley, 2012; Ooishi et al., 2012). It would seem that estrogen should, therefore, enhance performance on the MWM, which is dependent upon hippocampal function and synaptic plasticity; however, this is not the case. Chesler and Juraska (2000) posit that the MWM impairment from estrogen and progesterone replacement in ovariectomized rats could be caused by increased synaptic noise, since estrogen enhances synaptic transmission in many types of neurons and brain regions. If increased synaptic noise were the mechanism for which estrogen impairs MWM performance, other agents that facilitate synaptic activity should then also impair MWM performance. Glutamate agonists (Pitkanen, Sirvio, MacDonald, Ekonsalo, & Riekkinen, 1995; Staubli, Rogers, & Lynch, 1994) enhance MWM performance, while inhibitory agents, such as GABA agonists (Brioni & Arolfo, 1992; Kant, Wylie, Vasilakil, & Ghosh, 1996; McNamara, dePape, & Skelton, 1993; McNamara & Skelton, 1991) and NMDA antagonists (McNamara & Skelton, 1993; Morris et al., 1986; Morris, Steele, Bell, & Martin, 2013; Upchurch & Wehner, 1990) impair MWM performance. Thus, the hypothesis that estrogen interferes with MWM learning by increased synaptic noise does not appear to be supported.

1.3.4 Cognitive navigation strategies are similar in virtual and real space

The use of virtual environments for the study of human navigation/place learning first appeared in the 1990s and has been shown to be a reliable and valid analog to navigation in real space. Despite the limitations of using virtual environments, such as the typically narrow field of view and the absence of vestibular and proprioceptive information, similar cognitive mapping strategies are used to navigate through virtual space. Ruddle, Payne, and Jones (1997) demonstrated that people can learn to navigate in a virtual building as accurately as in a real building and that the number of objects used for landmarks directly influences performance, thus indicating the use of a mapping strategy based on environmental cues. Additionally, learning to navigate a virtual representation of a real environment facilitates the ability to navigate in that real environment (Regian & Yadrick, 1994; Witmer, Bailey, Knerr, & Parsons, 1996).

Adaptations of the MWM in virtual environments have shown that humans depend upon the presence of distal environmental cues to find the hidden escape platform (Astur, Ortiz, & Sutherland, 1998; Jacobs, Laurance, & Thomas, 1997; Jacobs, Thomas, Laurance, & Nadel, 1998; Sandstrom, Kaufman, & Huettel, 1998; Thomas, Hsu, Laurance, Nadel, & Jacobs, 2001). In one of the first published studies using a computerized virtual version of the MWM, Jacobs and colleagues (1997) found that across 10 acquisition trials, path length and time to find the hidden platform decreased, and the subjects spent the majority of the time searching the correct quadrant during a probe trial in which the platform was removed. After testing, the subjects were asked how they were able to find the platform. All subjects who indicated that they knew the location of the hidden platform reported that they used the distal environmental cues and their relations to orient their search. In a follow-up study by Jacobs et al. (1998), after subjects underwent acquisition under normal conditions, test phases were administered in which the distal environmental stimuli were removed or were swapped around. Under both of these conditions the subjects were unable to find the location of the hidden platform even though they had previously learned its location. These findings are consistent with the mapping strategy used by rodents and fit within the framework of cognitive mapping theory (Nadel, 1991), which states that when an organism explores a novel environment, a cognitive map is formed consisting of information about specific objects and how they spatially relate to each other. In the VWM, subjects who form these cognitive maps based on the distal cues find the hidden platform during acquisition trials. Removing a large number of these cues or significantly altering their spatial location renders the previously formed cognitive map invalid, and thus they are unable to find the hidden platform (Nadel et al., 1999).

1.3.5 Brain regions associated with virtual water maze

The involvement of the hippocampus in finding the hidden platform on the MWM is well established in rodents. In humans, the necessity of hippocampal involvement in place learning/navigation has also been demonstrated. In a study of patients with epilepsy who had

undergone hippocampal resections, severe impairments in the ability to learn the location of the hidden platform in the VWM were found (Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002). No differences in cued-navigation were found, and these effects were evident regardless of the side of the hippocampal resection. Amnesic patients with hippocampal damage also show selective impairment in place learning on the VWM (Goodrich-Hunsaker, Livingstone, Skelton, & Hopkins, 2010). Damage to the hippocampus has also impaired spatial navigation in other tasks that require navigation through virtual space (Bohbot et al., 1998; Maguire et al., 1998; Spiers et al., 2001).

The use of functional MRI has revealed a network of brain activations that interact with the hippocampus during place learning/navigation in the VWM. Hsu, Ryan, Nadel, Thomas, and Jacobs (2000) found brain activations in bilateral hippocampus, posterior parietal cortex, motor and pre-motor cortices, and the cerebellum while observing an experimenter completing the VWM. Another study using an analogue of the VWM required participants to navigate to the location of a visible pole (Parslow et al., 2004). The visible pole was then removed, and subjects had to navigate to its location using only the environmental cues available on the walls. Under a third condition, the wall cues were rotated between each trial, thus forcing the participants to use a directional strategy rather than a mapping strategy based on distal cues. In the condition where the wall cues remained stationary, activations were found in bilateral hippocampus and parahippocampal gyrus, frontal regions, precentral motor regions, parietal cortex, and the cerebellum. Under the condition in which the wall cues rotated between trials, a similar set of activations were found, however, no hippocampal/parahippocampal activations were present, indicating that these regions are activated during place learning and navigation only when distal environmental cues can be used. The frontal activations found are likely parts of the executive/planning aspects of the task, although this has not been formally investigated. There is evidence that the parietal cortex activations seen in these studies is involved in calculating the proper orientation required to find the platform. Using a version of the VWM in which locating a

hidden coin could only be achieved by using single local cues to determine which direction to go, the parietal cortex showed strong activations compared to a control task using allocentric or distal cues (Rodriguez, 2010).

These data from the lesion and functional imaging studies demonstrate that hippocampal function is necessary for learning and navigating to locations based on how those locations are spatially related to environmental cues. These studies are consistent with the animal literature and also from other studies of spatial navigation. Maguire, Frith, Burgess, Donnett, and O'Keefe (1998) found right hippocampal activation while subjects navigated a virtual maze using positron emission tomography. They also found bilateral activation of the hippocampus and parahippocampal regions were also found when comparing successful versus unsuccessful navigation of a large-scale virtual environment. Gray matter volume in the hippocampus has also been shown to correlate with the ability to navigate mazes (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Brown, Whiteman, Aselcioglu, & Stern, 2014), and age-related declines in hippocampal volume correlate with reduced hippocampal activation during navigation and performance on spatial learning tasks (Moffat, Elkins, & Resnick, 2006). Further studies that examine functional activation patterns and connectivity specifically using the VWM are needed to help determine the role of regions other than the hippocampus in spatial navigation.

1.3.6 Human sex differences on the virtual water maze

In the rodent literature there is a consistent finding that males perform better on the MWM than females and that this advantage appears to be related to the organizational effects of testosterone on the hippocampus early in development and the activational effects of natural fluctuations of estrogen and progesterone in females. The first study to compare men and women on the VWM found a large and reliable sex difference by which men performed better on three different versions of the task (Astur et al., 1998). The first version was modeled after the standard MWM in which there is a series of acquisition trials in which the platform is hidden, followed by a probe trial where the platform is removed, and lastly, cued navigation trials where the platform is

visible. Men performed better than women during acquisition and on the probe trial with effect sizes ranging from 0.5 to 1.0. No differences were detected during cued navigation. In an attempt to reduce the large between-sex effect sizes, a second version of the task was then created to make it more apparent that the platform does not move since during debriefing of the first experiment, the participants who performed poorly reported that they believed the platform was moving from trial to trial. This task was administered to a new group of participants where acquisition trials consisted of alternating visible and hidden platforms. Again, men found the platform faster than women when it was hidden, but no differences when it was visible were detected. A third version was then administered where the instructions were changed so that they explicitly told the participants that the platform does not move and that they should use the scenery and landmarks of the room. Again, men were faster during acquisition to locate the platform and spent more time in the quadrant where the platform was located during probe trials than women. Differences in experience with computer games were found between the sexes, but in a regression analysis, sex predicted performance over and above that predicted by computer game experience in all three experiments (Astur et al., 1998). This study found a large sex difference on the VWM that demonstrated that women perform worse than men on the VWM when they have to rely on a strategy of associating distal wall cues to the location. It was not clear if women would perform better if another strategy was available.

Sandstrom and colleagues (1998) examined sex differences on the VWM and altered the types of cues that were available to use to find the platform. A training phase with a visible platform took place in a trapezoidal room with four object landmarks placed in the room. This allowed the participants to utilize the room geometry (trapezoidal shape), the landmarks, or a combination of both as cues. A testing phase in which the platform was hidden was then performed under one of three conditions: (1) a stable landmark condition, where the location of the landmark remained the same but the room was now octagonal, thereby removing geometry cues; (2) a geometric condition, where the trapezoidal room was used, but all the landmarks were removed; and (3) a

random landmark condition, where the trapezoidal room was used, but the landmarks were placed in random places trial by trial. Men performed better than women in the geometric and random landmark conditions, where the landmark cues were either removed or made unreliable. In the stable landmark condition, women performed equally well as men, indicating that women tended to rely on landmarks for spatial navigation, while men relied on both landmarks and geometric cues (Sandstrom et al., 1998).

The findings from the Sandstrom et al. (1998) study suggest that the large sex difference found in the Astur et al. (1998) study is possibly due to the unavailability of landmark cues. In the Astur et al. study, each wall did have an identifying picture on it, but these pictures were not salient enough to use as landmarks. The landmarks in the Sandstrom et al. study were three dimensional objects that were placed around the room, not only on the walls. A study using pupillometry and eye tracking during VWM training found that men tend to explore more space with their gazes, consistent with the strategy of trying to understand the interrelations among objects and the geometry of the environment (Mueller, Jackson, & Skelton, 2008). Women in the study had longer fixation times and increases in pupil diameter usually associated with memory processing, indicating that they were focusing on encoding the location of specific landmarks. Men also choose their initial direction during VWM trials sooner than women, a behavior consistent with the use of distal cues (Woolley et al., 2010). Studies using real-life navigation tasks which contain both geometric and landmark cues have also found a reliable male advantage and that men tend to rely on both proximal landmarks and geometry of the environment, while women are more dependent on landmarks (Beatty & Troster, 1987; Galea & Kimura, 1992; Johnson & Meade, 1987; Linn & Peterson, 1985).

In the rodent literature, testosterone enhances performance on the MWM. To date, only one study has been conducted examining the relation between testosterone and VWM performance in humans. Burkitt, Widman, and Saucier (2007) found that women with low endogenous testosterone performed worse on the VWM than women with high endogenous

testosterone. No differences were found between men with low or high endogenous testosterone, and women with high testosterone performed as well as both groups of men. These findings are consistent with the rodent literature in that female rats treated with testosterone perform better on the MWM (Roof & Havens, 1992). The lack of a difference in VWM performance between men with low or high testosterone indicates that adult endogenous testosterone levels do not affect spatial navigation. Testosterone during development, either prenatal/neonatal periods or puberty may be the source for the male advantage on spatial navigation. Newhouse, Newhouse, and Astur (2007) tested pre-pubertal children on the VWM found that the sex difference still existed, indicating that this difference does not reflect the effects of sex hormones at puberty. Animal studies have found that manipulating the sex hormone environment prenatally induces affects in adulthood (Isgor & Sengalaub, 1998; Lund & Lephart, 2001; Roof & Havens, 1992). Future studies in humans are needed to examine whether prenatal/neonatal testosterone is related to the sexual dimorphism on the VWM.

1.3.7 Prenatal alcohol and Morris Water Maze

Impaired spatial learning on the MWM as a result of prenatal alcohol exposure was first reported in 1987 (Blanchard, Riley, & Hannigan, 1987; Goodlett, Kelly, & West, 1987) and has since been replicated many times under a variety of exposure conditions. Impaired spatial learning on the MWM is specific to hidden platform conditions where the use of distal environmental cues is required (Kelly, Goodlett, Hulsether, & West, 1988) and this effect is persistent throughout development (Gianoulakis, 1990; Kelly et al., 1988; Minetti, Arolfo, Virgolini, Brioni, & Fulginiti, 1996). The effects of prenatal alcohol exposure on spatial learning can be attenuated by enhancing the postnatal environment (Christie et al., 2005; Hannigan, Berman, & Zajac, 1993) or through postnatal choline supplementation (Thomas, Biane, O'Bryan, O'Neill, & Dominguez, 2007). Timing and pattern of alcohol exposure has also been shown to determine the severity of the effect.

The pattern of prenatal alcohol exposure is one factor that has consistently been shown to influence outcome. When alcohol is administered in a concentrated form, such that it produces high BACs, disruptions to the central nervous system and behavior are much greater than when the same amount is given over a less extended period of time, such that BACs are relatively low (Bonthius & West, 1990; Kelly, Hulsether, & West, 1987; Kelly, Pierce, & West, 1987; Pierce & West, 1986a; Pierce & West, 1986b; West, Kelly, & Pierce, 1986). Two studies that directly compared patterns of exposure found that rats exposed to ethanol in a concentrated, binge-like manner were greatly impaired on the MWM, whereas those exposed to an equal amount of ethanol, but at a lower-level constant rate, showed minimal effects (Goodlett et al., 1987; Kelly et al., 1988). A concentrated exposure pattern is more likely to produce damage to the hippocampus, which MWM performance is dependent upon. Reductions in hippocampal cell count (Bonthius & West, 1990; Greene, Diaz-Granados, & Amsel, 1992), cell density, and total hippocampal volume (Livy, Miller, Maier, & West, 2003) are found when ethanol exposure is concentrated rather than when administered in a more protracted manner.

The timing of alcohol exposure has varied greatly among studies that have used the MWM as an outcome. MWM impairment has been observed when exposure is spread across the prenatal period (Blanchard et al., 1987; Gianoulakis, 1990) and when restricted to single prenatal days (Minetti et al., 1996) or single postnatal days (Pauli, Wilce, & Bedi, 1995; Tomlinson, Wilce, & Bedi, 1998) that are the rodent equivalent of the human third trimester. Some of the more robust impairments on the MWM from prenatal alcohol exposure occur during the brain growth spurt that takes place from postnatal days 4 thru 10 (Goodlett et al., 1987; Kelly et al., 1988). More specifically, there tends to be a temporal window of vulnerability during the latter part of the brain growth spurt. Goodlett and Peterson (1995) administered alcohol to three groups of rats either across the whole brain growth spurt, postnatal days (PND) 4-9, or during either the first half (PND 4-6) or second half (PND 7-9) of the growth spurt. Alcohol exposure across the brain growth spurt produced MWM impairments in both male and female rats. No differences in MWM performance

were found in the group that was exposed during the early part of the brain growth spurt. When alcohol was administered during the latter part of the brain growth spurt, significant impairment in place learning was detected among males only, suggesting that the latter phase of the brain growth spurt constitutes a sex-specific period of increased vulnerability. This male-specific period of increased vulnerability was not due to the activating effects of circulating levels of female hormones, since the same male specific effect was found in pre-pubertal rats (Goodlett & Peterson, 1995). A follow-up study found that the lack of the female difference from PND 7-9 exposure could be due to poorer performance among females in general (Johnson & Goodlett, 2002). One possibility for the male specificity of the PND 7-9 critical window is that the masculinization of the hippocampus from testosterone in rodents could occur during this time; however, no studies have yet tested this possibility. What is known is that the PND 4-9 period that represents the whole brain growth spurt appears to be an important window of susceptibility to prenatal alcohol-related insult to hippocampal-mediated spatial learning on the MWM.

1.3.8 Fetal alcohol spectrum disorders and the virtual water maze

Hamilton et al. (2003) has been the only study published thus far examining the effect of prenatal alcohol exposure on VWM performance. Eight males (9.5-16.5 years old) diagnosed with FAS were compared to eight adolescent males matched for age and ethnicity with no history of prenatal alcohol exposure. During training, the FAS group traveled farther than controls to navigate to the hidden platform and spent less time searching the correct quadrant during a probe trial. By contrast, the FAS group did not differ from the control group during cued navigation, indicating that the group differences in place learning were not attributable to motivational or visual-motor deficits. These findings are consistent with findings when examining effects of prenatal alcohol exposure on the Morris water maze in rodents and may be a possible marker of hippocampal damage and/or dysfunction. These findings also raise interesting questions about the source of the place learning deficits that were found in the FAS group.

This study is not the first to detect impairment in hippocampal-dependent learning in FASD. Uecker and Nadel (1998) found that children with FAS were impaired in their ability to remember the location of objects they had seen in a grid. The children with fetal alcohol syndrome were able to remember a similar number of objects as control children, but when asked to identify the spatial locations of these objects they did so less successfully than control children. Along with the substantial rodent evidence, these data would suggest that the hippocampus is particularly sensitive to prenatal alcohol exposure. However, from a structural standpoint, evidence for hippocampal damage in humans prenatally exposed to alcohol is conflicted. Hippocampal volume reductions have been detected in a number of studies (Lebel et al., 2011), but when total brain size is controlled for, the hippocampus was disproportionally smaller in three studies (Archibald et al., 2001; Nardelli, Lebel, Rasmussen, Andrew, & Beaulieu, 2011; Willoughby, Sheard, Nash, & Rovet, 2008) but not in five other studies (Astley et al., 2009; Coles et al., 2011; Joseph et al., 2014; Riikonen et al., 2005; Roussotte et al., 2011). Thus, these raise some questions about whether the findings reported by Hamilton et al. (2003) are related to macrostructural changes to the hippocampus. They do not rule out the possibility that there are fetal alcohol-related microstructural or neurochemical changes to the hippocampus that could be the cause for the dysfunction, however, there are no studies to date that have detected such effects in humans.

The place learning impairments in the Hamilton et al. (2003) study could also be related to altered testosterone levels in the FAS group. In rodents, prenatal alcohol exposure is associated with reductions in the prenatal and early postnatal testosterone surges (McGivern, Handa, & Redei, 1993; McGivern, Raum, Salido, & Redei, 1988) and lower testosterone levels that persist through the period following puberty have been detected (Udani, Parker, Gavater, & Thiel, 1985). In a recent study we found that prenatal alcohol exposure was correlated with higher salivary testosterone levels in 14-year-old male and female adolescents (Carter, Jacobson, Dodge, Granger, & Jacobson, 2014). In addition, Roof (1993) found that increasing testosterone

in male rats impaired performance on the MWM. Thus, it is possible the poorer performance of the FAS group in the Hamilton et al. study could be due to prenatal alcohol-related changes in testosterone levels. It should be noted that there has been one study of normal adults using the VWM that found no differences in performance based on low or high levels of endogenous testosterone in males (Burkitt et al., 2007). It is possible the testosterone levels in this study did not substantially deviate from the norm and that adults may be less sensitive to differing testosterone levels than are developing adolescents.

There are some methodological limitations of the Hamilton et al. (2003) study, which if addressed, could provide valuable insight into the nature of the place learning deficits that were detected. First, the Hamilton et al. study had a sample that consisted only of males. The authors pointed out that future studies should also explore whether factors, such as sex, would alter the results. They also wrote that, "alcohol-related impairments in virtual place learning may be difficult to detect in females with FAS due to relatively poor place learning observed in normal females within the age group studied here." This statement was written over 10 years ago and may have reflected the belief that the male advantage in VWM tasks was their greater experience with computer-generated environments via video games. It is likely that in the last 10 years, the difference between male and female experience with computer-generated environments has diminished, and thus the overall place learning performance differences may have also diminished. Additionally, inclusion of females in an analysis of the effects of prenatal alcohol exposure on the VWM should generate valuable information. Given our recent findings that prenatal alcohol exposure is associated with increased testosterone in male and female adolescents (Carter et al., 2014) and findings from animal studies that increased testosterone impairs place learning performance in males but enhances it in females (Roof, 1993), it could be hypothesized that boys prenatally exposed to alcohol will show place learning deficits, while exposed girls would either show no difference or a possible enhancement of place learning. If confirmed, these findings would indicate that alcohol-induced place learning deficits would be

partly attributable to effects on sex hormones and how they interact with the hippocampus during development.

The inclusion of less severe forms of FASD in a study of virtual place learning would also be informative. The Hamilton et al. (2003) study only included male adolescents with FAS, the most severe form of FASD, in which alcohol-related physical dysmorphic features and growth retardation are evident, behavioral problems are more severe, and general intellectual impairment most pronounced. The inclusion of non-syndromal alcohol-exposed children would make it possible to determine whether spatial navigation deficits constitute a behavioral marker of prenatal alcohol exposure. Additional controls are also needed to clarify whether place learning impairments primarily reflect prenatal alcohol exposure or are mediated by alcohol-related reduced IQ or other behavioral impairments.

In summary, findings on the effects of prenatal alcohol exposure on hippocampal-dependent spatial memory are very limited in humans. Additional studies are needed to expand on the findings from Hamilton et al. (2003) beginning with some of the suggestions reviewed above. Future studies should be focused on examining the effects on the VWM in samples that comprise the whole range of FASD and also include continuous measures of prenatal alcohol exposure so that timing and pattern of effects described in the extensive rodent literature can be corroborated. Developmental exposure to sex hormones should also be considered as a possible mediator of the effects of prenatal alcohol exposure on the VWM in future studies.

1.4 Aims

The following study will use VWM data from three cohorts: a large moderately alcohol exposed, prospective longitudinal cohort recruited in Detroit, MI, and two heavily alcohol exposed cohorts recruited in Cape Town, South Africa. Since there were only three children diagnosed with FAS in the Detroit Longitudinal Cohort, it will not be used to test the first hypothesis in which VWM performance is compared between the different FASD diagnostic groups.

The first aim is to test the hypothesis that poorer performance on the VWM will be seen across the range of FASD diagnoses, from the most severe (FAS) to the other less severe manifestations (PFAS and ARND) in boys and girls. Using two cohorts of heavily-exposed children from Cape Town, who were diagnosed for FAS and PFAS by expert dysmorphologists, the effects of FASD diagnosis on performance on the VWM will be examined. This study will expand upon the findings of Hamilton et al. (2003) in two ways. First, by including children with not only FAS, but PFAS and those who are heavily exposed but lack the facial dysmorphology, this study will be able to determine whether VWM deficits are apparent in children with less severe forms of FASD and are intermediary in performance. Second, by including girls as well as boys in all of the analyses, this study will be able to determine whether FASD-related VWM performance deficits are similar in both sexes or if there is an interaction effect between FASD and sex.

The second aim is to test the hypothesis that the pattern and degree of prenatal alcohol exposure, using continuous measures of alcohol during pregnancy, is associated with poorer performance on the VWM. This will be the first study to examine the relation between the amount and frequency of prenatal alcohol exposure to VWM performance in humans. This study will use three cohorts of children for whom detailed prospective histories of pregnancy drinking were ascertained to examine the relation between amount and pattern of prenatal alcohol exposure to VWM performance, while controlling for prenatal cigarette and drug exposure, maternal and sociodemographic characteristics, and child's IQ.

The third aim is to test the hypothesis that prenatal alcohol-related damage to the hippocampus impairs performance on the VWM. It is known from rodent studies that the hippocampus is particularly sensitive to the effects of prenatal alcohol exposure and that the MWM is dependent upon hippocampal function. In humans, however, the effects of prenatal alcohol exposure on the hippocampus are less clear. This study will test the hypothesis that

hippocampal volume mediates the relation between prenatal alcohol exposure and VWM performance.

The fourth aim is to test the hypothesis that alterations in testosterone may mediate the relation between prenatal alcohol exposure and VWM performance. We have recently shown that prenatal alcohol exposure is associated with increased salivary testosterone in both boys and girls (Carter et al., 2014), and previous literature has shown that testosterone levels can affect water maze performance in rodents. Therefore, it is possible that one of the pathways by which prenatal alcohol exposure can influence place learning is through alterations in testosterone. This study will test the hypothesis that testosterone mediates the relation between prenatal alcohol exposure and place learning.

CHAPTER 2 METHODS

2.1 Samples

2.1.1 Detroit Longitudinal Cohort

The Detroit cohort was recruited prospectively during pregnancy between September 1986 and April 1989 to assess the effects of moderate-to-heavy levels of prenatal alcohol exposure (Jacobson, Chiodo, Sokol, & Jacobson, 2002). All African-American gravidas were screened for alcohol consumption during their first prenatal visit to a clinic in a large urban maternity hospital in Detroit. All women who averaged at least 7 drinks per week (0.5 oz AA per day) at the time of conception and a random sample of 5% of the lower level drinkers and abstainers were invited to participate in the study. To reduce the risk that alcohol would be confounded with cocaine exposure, a group of heavy cocaine (≥ 2 days/week), light alcohol (< 7 drinks/week) users were also recruited. Infant exclusionary criteria were birth weight less than 1500 grams, gestational age less than 32 weeks, major chromosomal anomalies or neural tube defects, and multiparous births.

The children were assessed during infancy (S. Jacobson et al., 1993, 1994; J. Jacobson et al., 1994), and at 7.5 (Burden et al., 2005a, 2005b; Jacobson et al., 2004) and 14 years (J. Jacobson et al., 2011). 128 young adults from the cohort participated at the 19-year follow-up conducted between 2006 and 2008, which consisted of a 2-day laboratory visit to our laboratory at Wayne State University comprised of neurocognitive testing and psychiatric interviews. Some of the participants were invited to Vanderbilt University for neuroimaging. The data used for this study consisted of the 104 participants at the 19-year follow-up that completed the VWM.

2.1.2 Cape Town Longitudinal Cohort (CT-L)

This sample consists of 131 children (mean age = 10.2; SD = 0.9) assessed on the VWM while participating in a 10-year follow-up of the first prospective, longitudinal study of FAS (Jacobson et al., 2008). All children were born to Cape Coloured women living in Cape Town. The Cape Coloured, a mixed ancestry population, is composed of descendants of white

European, Malaysian, and Khoi African ancestors. The prevalence of FASD in the Cape Coloured population in the Western Cape Province of South Africa is among the highest in the world (May et al. 2013), and the prevalence of FAS has been estimated to be 18 to 141 times greater than in the United States (May et al., 2000). The high prevalence of heavy maternal drinking during pregnancy is a consequence of poor psychosocial and socioeconomic circumstances and the traditional *dop* system, in which farm laborers were paid, in part, with wine (Croxford & Viljoen, 1999). The *dop* system has since been outlawed, however, heavy alcohol consumption persists in both rural and urban sectors (Jacobson, Jacobson, Molteno, & Odenhaal, 2006).

Pregnant mothers of the children in this cohort were recruited between July 1999 and January 2002 at the antenatal clinic of a midwife obstetric unit that serves an economically disadvantaged, predominantly Cape Coloured population. Research nurses interviewed each gravida at the first antenatal visit (*M* = 19.3 weeks gestation, SD = 6.4). A timeline follow-back approach was used to determine the frequency and amount of drinking on a day-by-day basis (Jacobson et al., 2002; Sokol, Martier, & Ernhart, 1985) at recruitment and around time of conception. Any woman averaging at least 1.0 ounce of absolute alcohol (AA) per day, or reporting at least two incidents of binge drinking (5 standard drinks/occasion) during the first trimester were also invited to participate. Women who drank less than 0.5 ounces of AA per day and did not binge drink during the first trimester were also invited to participate. Women less than 18 years of age and those with serious health problems (e.g., diabetes, epilepsy, or cardiac problems) requiring treatment were not included as were religiously observant Muslim women whose religious practices prohibit alcohol consumption and might have resulted in their being disproportionately represented in the control group. During recruitment and at each visit, all mothers were also interviewed about their smoking (number of cigarettes per day) and drug use (days per month), including marijuana (“dagga”), cocaine, and methaqualone (“mandrax”).

2.1.3 Cape Town Cross-Sectional Cohort (CT-CS)

This sample consists of 62 8- to 12-year-old (*M* = 10.4; SD = 1.2) children also from the Cape Coloured community in Cape Town (S. Jacobson et al., 2011). All are right-handed because they were originally recruited to participate in neuroimaging studies (Dodge et al., 2009; Meintjes et al., 2010). Thirty-six are older siblings of participants in the CT-L cohort described above. The other 26 were recruited by screening all 8- to 12-year-old children from an elementary school in a nearby rural section of Cape Town with a very high incidence of alcohol abuse. Mothers were retrospectively interviewed using the same timeline follow-back approach about their use of alcohol, cigarettes, and drug use during the time they were pregnant with the study child.

2.2 Procedure

2.2.1 Assessment of maternal alcohol use during pregnancy

For the Detroit cohort, maternal alcohol consumption during pregnancy was assessed using the timeline follow-back approach to determine the frequency and amount of drinking on a day-by-day basis (Jacobson et al., 2002; Sokol et al., 1985). At the first prenatal visit, each mother was asked about her drinking on a day-by-day basis during a typical 2-week period around the time of conception, with recall linked to certain times of day and activities. At each follow-up prenatal clinic visit (*M* = 5.2; SD = 3.4), the mother was questioned about how much alcohol she had consumed during the past 2 weeks. Volume was recorded for each type of alcoholic beverage consumed and converted to absolute alcohol (AA), using multipliers developed by Bowman and colleagues (1975). The number of days that the mother drank was also recorded. These values were averaged across the prenatal visits to obtain three measures of pregnancy drinking: ounces of AA per day across pregnancy; ounces of AA per occasion across pregnancy; and frequency of drinking days across pregnancy.

For the CT-L cohort, the same timeline follow-back approach was used as in the Detroit cohort. At the recruitment interview, each mother was asked about her drinking during a typical 2-week period around the time of conception. If her drinking had changed since conception, she was also asked her current drinking during the past 2 weeks and when her drinking had changed.

At each follow-up antenatal visit, the mother was asked about her drinking during the previous 2 weeks. At the 1-month postpartum visit, the mother was asked about her drinking during a typical 2-week period during the latter part of her pregnancy. The same three summary measures used in the Detroit cohort were constructed for this cohort. In three cases of children diagnosed with full FAS who were born to mothers who denied drinking, the three summary alcohol measures were estimated using the median of the FAS group whose mothers did not deny drinking.

Biological mothers of the children in the CT-CS cohort were interviewed about their pregnancy drinking retrospectively during their laboratory visit, and the three across pregnancy drinking measures were constructed based on their recall.

2.2.2 FASD Diagnostic Clinic.

In September 2005, a clinic was organized by Drs. S. Jacobson, J. Jacobson, and C. Molteno, at which each child from both cohorts was independently examined for growth and FAS anomalies using a standard protocol (Hoyme et al., 2005) by two expert U.S.-based FAS dysmorphologists (H.E. Hoyme, M.D., and L.K. Robinson, M.D.) There was substantial agreement between the two dysmorphologists on their assessments of palpebral fissure length and philtrum and vermillion ratings based on the Astley and Clarren (2001) rating scales (*r* values = 0.80, 0.84, and 0.77, respectively). There was also substantial agreement between them and the Cape Town-based dysmorphologist (N. Khaole) (median *r* = 0.78) who evaluated 21 children from the CT-L cohort and 26 children from the CT-CS cohort who could not be scheduled for the clinic. FAS and PFAS diagnoses were subsequently determined at case conferences by HEH, LKR, SWJ, JLJ, and CDM. Based on the diagnoses from the case conferences, four groups were constructed for the analyses described below: FAS, PFAS, heavily exposed non-syndromal (HE), and controls.

2.2.3 Virtual Water Maze: Detroit and CT-CS cohort

The procedures of the VWM administered to the Detroit and CT-CS cohorts were those developed by Hamilton et al. (2003). All assessments including the VWM were administered by a Master's level psychologist who was blind regarding FASD group or maternal alcohol history. The

virtual environment was comprised of a circular pool centered in a square room. Four different rectangular objects of equal size were placed on each of the distal room walls. The platform occupied approximately 2% of the pool area and was located in the northeast quadrant of the pool. The virtual environment was displayed on a PC monitor with a 45-degree field of view. Forward movement was controlled by the up arrow key on the keyboard, and rotation was controlled by the left and right arrow keys. Backward and up/down movement was not possible.

The test was administered in three phases with a total time of approximately 30 minutes. The first phase, acquisition, was composed of five hidden-trial blocks, with four trials in each block. A 60-second time limit was allotted to find the platform for each trial. If the trial was not found after the time elapsed, the platform was made visible and a tone was sounded to inform the participant that the platform was visible. After locating the platform, participants remained on the platform for 5 seconds during which they could not leave the platform but were allowed to rotate around to view the environment. Afterwards, the display was removed, and a 2-second inter-trial interval was used. Starting locations for each trial were determined pseudo-randomly from one of four locations around the perimeter of the pool. All four of these starting locations were used once during each of the five blocks. Total time to reach the hidden platform was recorded for each trial.

The second phase, probe, consisted of a single 45-second trial in which the platform was removed from the environment. The starting location was selected randomly from one of two starting locations that were furthest from the location of the platform. Percentage of time spent in the platform quadrant was recorded as the dependent variable.

The final phase, cued navigation, consisted of two four-trial blocks in which the platform was raised slightly above the surface of the water so that it was visible.

During acquisition and cued navigation, participants were able to earn points for finding the platform more quickly in order to increase motivation and attention to the task. The points were only treated as a symbolic score and were not redeemable for other rewards. The number of points the participant could earn was inversely related to the latency to find the platform.

Participants were instructed to earn as many points as possible by quickly finding the platform. Each participant was also informed that the platform would always be in the same location relative to the distal cues and that the starting positions would be in different locations.

2.2.4 Computer-Generated (CG) Arena Water Maze: CT-L cohort

A Master's level psychologist, blind with respect to FASD status and prenatal alcohol history, administered all of the neurocognitive assessments including the Computer-Generated (CG) Arena (Thomas et al., 2001) water maze to each child. The CG Arena water maze is very similar to the VWM test. Like the version administered to the Detroit and CT-CS cohorts, the CG Arena water maze test is comprised of acquisition, probe, and cued navigation phases. The virtual environment was also comprised of a circular pool centered in a square room. A single image was placed on two of the walls, the other two walls had three distinct images. The platform was located in the northwest quadrant. Movement was controlled using the up (forward) and down (backward) arrow keys, and rotation was controlled by the left and right arrow keys on the keyboard.

At the start of the task, the child was placed in the 'virtual waiting room,' which was similar to the testing environment except that there was no platform and no objects on the walls, instead each wall was a different color. While in the waiting room, the examiner instructed the children to practice swimming to each of the colored walls. Next, there was a practice phase consisting of four trials with the platform visible, and no room cues available. The child was told to get to the platform as quickly as possible and that the platform would always be in the same location. After each trial, the child was transported back to the virtual waiting room.

Two versions of this task that varied only in the amount of time given per trial were administered to different children in this cohort. The acquisition phase consisted of 16 trials with a hidden platform in the testing environment. In the first version, which was administered to 47 children, each trial had a time limit of 120 seconds. In the second version of the test, administered to 84 children, each trial was reduced to only 60 seconds, in order to make the test more

challenging. In both cases, the children were told that the platform is now hidden and that they need to find it and remember where it was located. They were informed that they can look at the pictures on the walls to help remember the location, and the platform would always be in the same location. Once the child found the platform, it became visible and 5 seconds were allotted to examine the environment. Time taken to find the hidden platform was used as the dependent variable.

The probe phase was a single trial during which the platform was removed. In the first version, this trial had a duration of 120 seconds; in the second version, a duration of 45 seconds, again reduced in order to make the task more challenging. During this trial the child was encouraged to keep searching for the platform. Percentage of time spent in the platform quadrant was used as the dependent variable.

Cued navigation consisted of four trials where the platform was visible, and only a single wall object was present. In the first version, 120 seconds were allotted per trial, in the second version, 60 seconds per trial. Total time taken to reach the platform was used as the dependent variable.

Each of the two versions of the task administered to this cohort will be analyzed separately. The name ‘CT-L CG-1’ will refer to the first version of the task that was comprised of 120-second trials. The name ‘CT-L CG-2’ will refer to the second version comprised of 60-second acquisition trials, and a 45-second probe trial.

2.2.5 IQ

Each participant in the Detroit cohort was administered the Wechsler (1991) Intelligence Scales for Children, 3rd edition (WISC-III) during their 14-year follow-up visit.

For the CT-L cohort, the Wechsler Intelligence Scales for Children-IV (WISC-IV; Wechsler, 2004) was administered during the 10-year laboratory visit. IQ for the CT-CS cohort was estimated based on Sattler’s (1992) formula for Short Form IQ using 7 of 10 subtests from the Wechsler Intelligence Scales for Children-III (WISC-III; Wechsler, 1991)—Similarities, Arithmetic, Digit

Span, Symbol Search, Coding, Block Design, and Picture Completion, as well as one subtest from the WISC-IV—Matrix Reasoning. Validity coefficients for the Sattler Short Form IQ based on 5 or more subtests consistently exceed $r = 0.90$.

2.2.6 MRI acquisition and image analysis.

For the CT-CS cohort, all scans were acquired using a 1.5T Magnetom Symphony MRI scanner (Siemens Medical Systems, Erlangen, Germany). High-resolution anatomical images were acquired in the saggital plane using a three-dimensional inversion recovery gradient echo sequence (72 slices, TR = 1900 ms, TE = 3.93 ms, TI = 1100 ms, slice thickness 2mm), 250 x 250 mm field of view, resolution 1.4 x 1.0 x 2 mm. Total gray matter, white matter, CSF volumes, and hippocampal volumes were extracted using FreeSurfer V5.1.0.

All children in the CT-L cohort were scanned on a 3T Allegra MR scanner (Siemens, Erlangen Germany). A magnetization-prepared rapid gradient echo structural image was acquired in a sagittal orientation with the following parameters: TR = 2300 ms, TE = 3.93, TI = 1100 ms, 160 slices, flip able 12 degrees, voxel size = 1.3 x 1.0 x 1.0 mm^3, and scan time = 6:03 minutes. FreeSurfer V5.1.0 was used to extract total gray, white, and CSF volumes as well as hippocampal volumes.

2.2.7 Salivary testosterone collection

Five-milliliter saliva samples were obtained for testosterone analysis from a subset of those seen at the 14-year visit of the Detroit cohort, yielding testosterone data for 37 participants for these analyses (Carter et al., 2014). The samples were collected in the morning upon the participant's arrival at the laboratory and at least 1 hour since their last meal. Samples were frozen at -70 degrees Celsius and subsequently sent in batches on dry ice via overnight delivery to Salimetrics Laboratories (State College, PA), where they were assayed for testosterone using an enzyme immunoassay specifically designed for use with saliva according to the manufacturer's recommended protocol with modification (Granger, Schwartz, Booth, & Arentz, 1999; Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). The assay's test volume is 50 ul with a range of

sensitivity from 1.5 to 360 pg/ml, and the average inter and intra-assay coefficients of variation were <15 and 5%, respectively. All saliva samples were assayed in duplicate, and the average of the duplicates was used.

2.2.8 Selection of confounding variables

Correlational analysis was used to determine which control variables were adjusted for statistically as potential confounders. A control variable cannot be the true cause of an observed deficit unless it is associated with both the exposure and the outcome (Schlesselman, 1982). Therefore, a relation with either exposure or outcome can be used as the criterion for statistical adjustment. In these studies, control variables were selected based on their relation to the outcome, which has the additional advantage of increasing precision by also including covariates unrelated to exposure (Kleinbaum, Kupper, & Muller, 1988). All control variables that were even weakly related to each outcome ($p < 0.10$) were controlled for statistically.

For the Detroit cohort, eight control variables were assessed as potential confounders: participant age at testing; self-reported current alcohol use (AA/day), smoking (cigarettes/day), and marijuana use (days/month); mother's age at delivery; maternal smoking (cigarettes/day), cocaine (days/month), and marijuana (days/month) use during pregnancy. For both Cape Town cohorts, there were four control variables that were assessed for consideration as potential confounders: age at testing, mother's age at delivery, marital status, and maternal cigarette smoking during pregnancy (cigarettes/day). Prenatal exposure to marijuana, cocaine, and methaqualone (days/month) were also considered as potential confounders provided there were a sufficient number of children exposed to produce a viable distribution with sufficient variance. If there were not enough cases of prenatal exposure to a particular drug, it was not controlled for in the statistical analysis. Instead, secondary analyses were performed excluding the exposed children to determine if the prenatal drug exposure had any influence on how FASD diagnosis/prenatal alcohol exposure related to the outcomes. Sex was treated as a between-

subjects factor in all ANCOVAs performed and included as a control variable in all multiple regression analyses.

2.2.9 Informed consent

Human subjects approval was obtained from the Wayne State University and University of Cape Town Faculty of Health Sciences ethics committees. For both cohorts, informed consent was obtained from each mother at recruitment and at the follow-up visits, and assent was obtained from the child. Children received a small gift; mothers received compensation consistent with guidelines from the ethics committees and a photo of the child. Mothers and children were transported to and from the University of Cape Town Child Development Research Laboratory in a research-dedicated van by the research driver and nurse and given breakfast, a snack, and lunch during the visit.

2.3 Data Analysis

The first hypothesis is that poorer performance on the VWM will be seen across the range of FASD diagnoses, from the most severe (FAS) to the other less severe manifestations (PFAS and HE) in boys and girls. This hypothesis will be tested using the two heavily exposed cohorts of children from Cape Town. Given differences in recruitment and VWM procedures administered to each of the cohorts as described above, the effects of FASD diagnosis on VWM performance will be tested separately in individual analyses for each of the two cohorts.

To test the hypothesis that FASD diagnosis is related to poorer performance on the VWM, two endpoints will be examined for each cohort. First, performance in the acquisition phase will be assessed using repeated-measures ANCOVA. For the CT-CS cohort, five four-trial acquisition blocks were created based on average trial time to find the platform. Following Hamilton et al. (2003), blocks 2 and 3 were combined as were blocks 4 and 5. Thus, the dependent variable during the acquisition phase is average trial time, with block (Block 1 vs. Blocks 2-3 vs. Blocks 4-5) as the within-subjects factor. FASD diagnosis and sex were the between-subjects factors. For the CT-L cohort, the 16 learning trials were combined into four blocks of four trials. Mean trial time

for each of the four blocks was the dependent variable, block the within-subjects factor, and FASD diagnosis and sex the between-subjects factors. Any potential confounder that met the criterion described above was entered as a covariate. The hypothesis that FASD diagnosis is associated with poorer place learning was determined by the presence of a main effect of FASD diagnosis and/or an interaction effect between FASD diagnosis and learning block. Mean trial time was expected to decrease across the learning blocks. In controls, the decrease in mean trial time across blocks is expected to be greater than in those exposed to alcohol. Because of the sexual dimorphism in place learning seen in both rodent and human studies, main effects and/or interactions with sex were expected. To explore these, separate repeated-measures ANCOVAs with block as the within-subjects factor and FASD diagnosis as the between-subjects factor were performed for each sex.

Secondly, VWM performance was also assessed by examining the percentage of time spent in the platform quadrant during the probe trial as the dependent variable in an ANCOVA with FASD diagnosis and sex as the between-subjects factors. The hypothesis that FASD diagnosis was associated with poorer place learning was determined by the presence of a main and/or interaction effect of FASD diagnosis. It is expected that controls will spend significantly more time in the platform quadrant than the exposed groups.

To determine if the effects of FASD diagnosis on VWM performance was mediated by reduced intellectual function typically observed in FAS and PFAS, a second set of analyses were run with the addition of IQ as a covariate to see if the FASD effect persisted.

The second hypothesis that prenatal alcohol exposure is associated with performance on the VWM was tested using all three cohorts described above. To test this hypothesis that prenatal alcohol exposure is related to poorer VWM performance, the same two endpoints were used, mean trial across block during acquisition, and percentage of time spent in the platform quadrant during the probe trial. Repeated measures ANCOVA was used with block as the within-subjects factor and sex entered as a between-subjects factor, and all potential confounders that met the

criterion were entered as covariates. Each of the three prenatal alcohol exposure variables (AA/day, AA/occasion, and frequency) was entered as covariates in separate analyses. A significant main effect and/or interaction of alcohol indicated that prenatal alcohol exposure is related to VWM performance, and further analyses to examine the nature of the effect were conducted. Any significant alcohol measure was then categorized and entered as a between-subjects factor in a follow-up ANCOVA. To determine if the effects of prenatal alcohol exposure on place learning were related to reduced global intellectual function a second set of analyses was run with the addition of IQ as a covariate.

Multiple regression analysis was used to determine the relation between prenatal alcohol exposure and percentage of time spent in the platform quadrant during the probe trial. Prenatal alcohol exposure, sex and potential confounding variables that met criterion were entered as predictors. IQ was entered at the second step. Standardized regression coefficients were evaluated to determine the relation between prenatal alcohol exposure and probe trial performance.

To test the hypothesis that prenatal alcohol-related damage to the hippocampus impairs performance on the VWM, structural MRI data obtained from the CT-L and CT-CS cohorts was used to examine the relation between hippocampal volume, VWM performance, and FASD diagnostic group/prenatal alcohol exposure.

Correlation analysis was used to determine if hippocampal volume is associated with prenatal alcohol exposure and place learning. Due to reduced head circumference and brain volume among the heavily exposed, both raw hippocampal volume and hippocampal volume adjusted for total brain volume were examined. Mediation by hippocampal volume of the relation between FASD diagnosis and/or prenatal alcohol exposure on VWM performance was tested using the MEDIATE macro in SPSS (Hayes & Preacher, 2014), a multivariate extension of the Sobel (1982) method. This macro allows the use of multi-categorical independent variables and multiple mediators. Models were created using FASD diagnostic group or prenatal alcohol

exposure as independent variables (IV), virtual water maze performance as the dependent variable (DV), and hippocampal volume as the potential mediating variable (MV). Mediation was determined based on the size of the indirect effects, which are the product of the unstandardized regression coefficients for the direct effects of IV on MV, and MV on DV. Statistical significance of the indirect effects was determined based on 95% bootstrap percentile confidence intervals. Indirect effects were deemed statistically different from zero when the confidence intervals did not overlap with zero.

To test the hypothesis that alterations in testosterone may mediate the relation between prenatal alcohol exposure and VWM performance, salivary testosterone levels obtained from a subset of the Detroit cohort were used. Correlation analyses were used to examine whether salivary testosterone levels were associated with VWM performance. All analyses were run separately for boys and girls. If an effect of testosterone on VWM was detected, mediation analyses as described above were performed.

CHAPTER 3 RESULTS

3.1 Sample characteristics

3.1.1 CT-L cohort

Table 1 presents the sample characteristics for the 131 children administered the CG Arena water maze test. Mothers in the FAS group were older than those in the PFAS, HE, and control groups (all *p*s < .05). Primary caregivers in the PFAS group were less educated than those in the FAS, HE, and control groups (all *p*s < .05). The proportion of married caregivers was not significantly different between the diagnostic groups. Mothers in the control group smoked less during pregnancy than mothers in the PFAS ($p = 0.06$) and HE ($p < .05$) groups. Twelve (9.2%) mothers reported smoking marijuana during pregnancy ($M = 2.4$ days/week), but there were no differences between diagnostic groups. Only one (0.8%) mother reported using cocaine during pregnancy (2.6 days/week), and three (2.3%) mothers reported using methaqualone during pregnancy ($M = 1.3$ days/week).

On average, mothers in the FAS group drank the most (all *p*s < .01), and PFAS and HE mothers drank more per day than control mothers (all *p*s < .001). Similarly, mothers in the FAS group drank more per occasion than the other three groups (all *p*s < .05), and the PFAS and HE drank more per occasion than the control group (all *p*s < .001). FAS and PFAS mothers drank more frequently than those in the HE and control group, and those in the HE group drank more frequently than controls (all *p*s < .05). All but two (3.7%) of the mothers in the control group abstained from alcohol during pregnancy. The two mothers in the control group that did drink during pregnancy drank minimally ($M = 1.8$ drinks per occasion across 4.2 drinking days).

Children in the PFAS group were younger at testing compared to the other three groups (all *p*s < .10). There were no between-group differences in sex composition. As expected, children in the FAS and PFAS groups had significantly lower IQs than children in the HE and control groups (all *p*s < .01), but there no IQ differences between the FAS and PFAS group, and no differences between the HE and control groups.

Because of the relatively low number of children in the FAS and PFAS groups, these two groups were combined for the subsequent analyses.

3.1.2 CT-CS cohort

Table 2 presents the sample characteristics for the 62 children who completed the VWM. No differences between diagnostic groups were detected for maternal age, SES, or marital status. The primary caregivers of the FAS and HE groups had completed significantly fewer years of school than the control group (all *p*s < .05). Mothers of HE children smoked more cigarettes during pregnancy than mothers of the controls ($p < .05$). Only one (1.6%) mother reported smoking marijuana during pregnancy, and no mothers reported cocaine or methaqualone use. There were no differences between the exposed groups, FAS, PFAS, and HE on any of the three alcohol exposure measures; however, per design, all three groups drank more than controls (all *p*s < .01).

There were no between-group differences in child age at testing or sex. Children in the FAS group had significantly lower IQs than children in the PFAS, HE, and control groups (all *p*s < .05), and children in the HE group has lower IQs than control children ($p < .05$).

Because of the relatively low number of children in the FAS and PFAS groups, these two groups were combined for the subsequent analyses.

3.1.3 Detroit Longitudinal Cohort

Table 3 presents the sample characteristics for the Detroit Longitudinal Cohort. The sample was comprised of young adults who grew up in economically disadvantaged households. Although 44.2% of the households at the 14-year follow-up visit were in levels IV and V of the Hollingshead (2011) SES scale (semi-skilled or unskilled workers), 75.0% of the sample's primary caregivers had completed 12 years of education. During pregnancy, 77.8% of the sample was exposed to alcohol, 59.6% to cigarettes, 33.7% to marijuana, and 35.6% to cocaine. IQ scores at age 14 were in the range commonly associated with inner-city, African American samples (Sattler, 1992). At the time of the 19-year follow-up, 16.2% of the sample smoked cigarettes, and 33.6%

used marijuana. While 33.7% currently drank alcohol, 11 (31.4%) of these consumers reported binging in the past month, and 7 (20.0%) reported drinking at least once per week.

3.2 Virtual Water Maze Performance

3.2.1 CT-L cohort—CT-L CG-1

Figure 1 presents the mean time to find the hidden platform across the four blocks during acquisition in the 120-s trial version of the task. Repeated-measures ANOVA revealed a significant decrease in time to locate the hidden platform across the four blocks ($F(3, 149) = 4.94$; $p < .01$; Fig. 1). Post-hoc analyses revealed significant decrements in time between blocks 2 and 1 ($p < .05$), and the times in both blocks 3 and 4 was significantly less than in blocks 1 and 2 (ps < .001). During the probe trial where the hidden platform is removed, participants spent most of the time searching the northwest quadrant where the hidden platform was located during place learning trials (Fig. 2). Paired-samples *t*-tests showed that time spent in the northwest platform quadrant was significantly greater than time spent in the northeast ($t(46) = 6.43$; $p < .001$), southeast ($t(46) = 7.60$; $p < .001$), and southwest ($t(46) = 7.54$; $p < .001$) quadrants.

3.2.2 CT-L cohort—CT-L CG-2

Figure 3 presents the mean time to find the hidden platform across the four blocks during acquisition in the 60-sec trial task. Repeated-measures ANOVA also revealed a significant decrease in time to locate the hidden platform across the acquisition blocks ($F(3, 249) = 17.98$; $p < .001$; Fig. 3). Post-hoc analyses revealed significant decrements in time between blocks 2 and 1 ($p < .05$), and the times in both blocks 3 and 4 was significantly less than in blocks 1 and 2 (ps < .001). During the probe trial, participants also spent most of the time searching the northwest quadrant where the hidden platform was located during acquisition (Fig. 4). Similarly, paired-samples *t*-tests showed that time spent in the northwest platform quadrant was significantly higher than time spent in the northeast ($t(83) = 5.53$; $p < .001$), southeast ($t(83) = 6.28$; $p < .001$), and southwest ($t(83) = 4.68$; $p < .001$) quadrants.

3.2.3 CT-CS cohort

Figure 5 presents the mean time to find the hidden platform across the three blocks during acquisition. Repeated-measures ANOVA revealed a significant decrease in time to locate the hidden platform across the three blocks ($F(2, 122) = 8.68$; $p < .001$; Fig. 3). Post-hoc analyses revealed significant decrements in time between blocks 2 and 1 ($p < .05$), blocks 1 and 3 ($p < .001$), and blocks 2 and 3 ($p < .05$). Figure 6 presents the percentage of time spent searching each quadrant during the probe trial. During acquisition, the platform located in the NE quadrant. Paired-samples *t*-tests showed that there were no differences in time spent in the northeast platform quadrant compared to the northwest ($t(49) = 1.43$; $p > .10$), southeast ($t(49) = 0.15$; $p > .10$), and southwest ($t(49) = 0.12$; $p > .10$) quadrants.

3.2.4 Detroit Longitudinal cohort

Figure 7 presents the mean time to find the hidden platform across the three blocks during acquisition. Repeated-measures ANOVA revealed a significant decrease in time to locate the hidden platform across the four blocks ($F(2, 206) = 37.52$; $p < .001$; Fig. 3). Post-hoc analyses revealed significant decrements in time between all blocks ($p < .001$). Figure 8 presents the percentage of time spent searching each quadrant during the probe trial. Paired-samples *t*-tests revealed that the percentage of time spent in the northeast platform quadrant was significantly greater than the time spent in the northwest ($t(103) = 3.91$; $p < .001$), southeast ($t(103) = 4.58$; $p < .001$), and southwest ($t(103) = 5.37$; $p < .001$) quadrants.

3.3 Effects of FASD diagnosis on virtual water maze performance

3.3.1 CT-L cohort—CT-L CG-1

A repeated measures ANCOVA was conducted to determine the effect of FASD diagnosis on virtual water maze performance during acquisition. FASD diagnosis (FAS/PFAS vs. HE vs. Control) and sex were between-subject factors and block (1 vs. 2 vs. 3 vs. 4) was the within-subjects factor. Primary caregiver's marital status and prenatal cigarette exposure were included as covariates. A significant main effect of sex [$F(1, 36) = 8.60$; $p < .01$] was detected. Post-hoc analyses revealed that across all blocks, girls took significantly longer to locate the hidden

platform than boys (*M* = 48.0 vs. 31.2 seconds, respectively). There were no significant main effects or interaction effects of FASD diagnostic group. No significant main effect or interaction with block was detected either. Figure 9 presents the covariate adjusted mean trial times by block for each of the FASD diagnostic groups by sex.

To examine the effect of FASD diagnostic group on performance during the probe trial, ANCOVA was performed with percentage of time in the platform quadrant as the dependent variable. Both FASD diagnostic group and sex were entered as between-subjects factors. No potential confounders met the criterion to be included as covariates. The main effect of sex was not significant [$F(1, 41) = 2.07$; n.s.], nor was the main effect of FASD diagnostic group [$F(2, 41) = 1.27$; *n.s.*]. No sex X FASD diagnostic group interaction effect was detected [$F(2, 41) = 0.48$; n.s.]. Figure 10 presents the adjusted means for each of the diagnostic groups.

ANCOVA was performed to assess the effects of FASD diagnostic group on performance during cued-navigation. Mean trial time during cued-navigation blocks was the dependent variable, and FASD diagnostic group and sex were the between subject factors. Prenatal cigarette exposure was entered as a covariate. No significant main or interaction effects involving FASD diagnostic group (Figure 11) or sex were found.

3.3.2 CT-L cohort—CT-L CG-2

A repeated measures ANCOVA was conducted to determine the effect of FASD diagnosis on virtual water maze performance during place learning. FASD diagnosis (FAS/PFAS vs. HE vs. Control) and sex were between-subject factors and block (1 vs. 2 vs. 3 vs. 4) was the within-subjects factor. Prenatal marijuana exposure and age at testing were included as covariates. No significant main or interaction effects of sex or FASD diagnostic group were detected. Figure 12 presents the covariate adjusted mean trial times by block for each of the FASD diagnostic groups by sex.

To examine the effect of FASD diagnostic group on performance during the probe trial an ANCOVA was performed with percentage of time in the platform quadrant as the dependent

variable. Both FASD diagnostic group and sex were entered as between-subjects factors, and mother's age at delivery, and prenatal marijuana exposure were entered as a covariates. The main effect of sex was not significant [$F(1, 76) = 0.72$; n.s.], nor was the main effect of FASD diagnostic group [$F(2, 76) = 0.58$; *n.s.*]. No sex X FASD diagnostic group interaction effect was detected [$F(2, 76) = 0.21$; n.s.]. Figure 13 presents the adjusted means for each of the diagnostic groups.

A repeated-measures ANCOVA was performed to assess the effects of FASD diagnostic group on performance during the cued-navigation trials where the platform is visible. Mean trial time during cued-navigation blocks was the dependent variable, and FASD diagnostic group and sex were the between subject factors. Age at testing, prenatal cigarette exposure, and mother's age at delivery were entered as covariates. The main effect of sex was not significant [$F(1, 75) = 2.60$; n.s.], nor was the main effect of FASD diagnostic group [$F(2, 75) = 0.55$; *n.s.*]. No sex X FASD diagnostic group interaction effect was detected [$F(2, 75) = 1.52$; n.s.]. Figure 14 presents the adjusted means for each of the diagnostic groups.

3.3.3 CT-CS cohort

A repeated measures ANCOVA was conducted to determine the effect of FASD diagnosis on VWM performance during acquisition. FASD diagnosis (FAS/PFAS vs. HE vs. Control) and sex were between-subject factors and block (1 vs. 2-3 vs. 4-5) was the within-subjects factor. Age at testing and mother's age at delivery were included as covariates. Significant main effects of FASD diagnosis [$F(2, 54) = 5.65$; $p < .01$] and sex [$F(1, 54) = 8.50$; $p < .01$], and a marginally significant sex X FASD interaction effect [$F(2, 54) = 3.00$; $p = .058$] were detected. No significant main effect or interaction with block was found. Post-hoc analyses revealed that across all blocks, girls took significantly longer to locate the hidden platform than boys ($p < .01$), and those in the FAS/PFAS group took significantly longer to locate the hidden platform than the HE ($p < .01$) and control ($p < .01$) groups. These effects remained unchanged after removal of the one child exposed to marijuana during pregnancy. When IQ was added to the model, the main effects of

FASD diagnosis [$F(2, 53) = 3.60$; $p < .05$] and sex [$F(1, 53) = 10.82$; $p < .01$] and the sex X FASD interaction effect [$F(2, 53) = 2.97$; $p = .060$] persisted.

Figure 15A presents the covariate-adjusted mean trial times for each block by FASD diagnosis for boys. For each block, ANCOVAs were performed to determine if mean trial time differed by FASD diagnosis. No significant between-group differences were detected for block 1 [$F(2, 23) = 0.60$; n.s.]. Significant group differences were detected on both blocks 2-3 [$F(2, 23) = 6.55$; $p < .01$] and blocks 4-5 [$F(2, 23) = 8.18$; $p < .01$]. On blocks 2-3, the FAS/PFAS group took significantly longer to find the hidden platform than the HE ($p < .01$) and the control groups ($p < .001$), while on blocks 4-5 both the FAS/PFAS ($p < .01$) and HE ($p < .01$) groups took significantly longer to locate the hidden platform than controls.

Figure 15B presents the covariate-adjusted mean trial times for each block by FASD diagnosis for girls. As with the analysis on the boys described above ANCOVAs were performed for each block to determine if mean trial time differed by FASD diagnosis. No significant between-group differences were detected for block 1 [$F(2, 29) = 1.85$; n.s.] nor blocks 2-3 [$F(2, 29) = 0.80$; n.s.]. Significant group differences were detected on blocks 4-5 [$F(2, 29) = 4.00$; $p < .05$], in which the FAS/PFAS group took significantly longer to find the hidden platform than the HE ($p < .01$) and the control groups ($p < .05$).

To examine the effect of FASD diagnostic group on performance during the probe trial an ANCOVA was performed with percentage of time in the platform quadrant as the dependent variable. Both FASD diagnostic group and sex were entered as between-subjects factors, and age at testing was entered as a covariate. The main effect of sex was not significant [$F(1, 55) = 0.17$; n.s.], whereas the main effect of FASD diagnostic group was marginally significant [$F(2, 55) = 2.43$; $p = .097$]. No sex X FASD diagnostic group interaction effect was detected [$F(2, 55) = 0.31$; n.s.]. Figure 16 presents the adjusted means for each of the diagnostic groups. Children in the HE group spent significantly less time searching the platform quadrant than control children

($p < .05$). The effect of diagnostic group was no longer significant after removing the one child exposed to marijuana or when IQ was added to the model.

ANCOVA was performed to assess the effects of FASD diagnostic group on performance during cued-navigation. Mean trial time during two cued-navigation blocks was the dependent variable, and FASD diagnostic group and sex were the between subject factors. Age at testing, primary caregiver's marital status, and prenatal cigarette exposure were entered as covariates. No significant main or interaction effects involving FASD diagnostic group were found (Figure 17). A significant main effect of sex was detected [$F(1, 53) = 8.91$; $p < .01$], in which girls took longer to navigate to the platform ($M = 13.2$ s) than boys ($M = 9.5$ s).

3.4 Effects of continuous measures of prenatal alcohol exposure on virtual water maze performance

3.4.1 CT-L cohort—CT-L CG-1

Repeated measures ANCOVAs were conducted to determine whether continuous measures of prenatal alcohol exposure are associated with performance during the acquisition phase. Sex was entered as a between-subject factor and block (1 vs. 2 vs. 3 vs. 4) was the within-subjects factor. Primary caregiver's marital status and prenatal cigarette exposure were included as covariates. Each of the three measures of prenatal alcohol exposure (AA/day, AA/occasion, and Frequency) was entered as a covariate in separate analyses. For AA/day, neither the main effect [$F(1, 39) = 0.16$; n.s.] nor the interaction with block were significant [$F(3, 117) = 1.26$; n.s.]. Similarly, for AA/occasion and frequency, neither the main effects ([$F(1, 39) = 0.18$; n.s.] and [$F(1, 39) = 0.96$; n.s.], respectively), nor the interactions with block were significant ([$F(3, 117) = 0.68$; n.s.] and [$F(3, 117) = 1.67$; n.s.], respectively).

Multiple regression analyses were performed to determine if prenatal alcohol exposure was associated with performance during the probe trial. Frequency of drinking was marginally associated with a decrease in time spent in the platform quadrant during the probe trial, but was no longer significant after control for sex (Table 4).

None of the three measures of prenatal alcohol exposure was related to mean trial time during cued navigation (Table 5).

3.4.2 CT-L cohort—CT-L CG-2

Repeated measures ANCOVAs were conducted to determine whether prenatal alcohol exposure is associated with acquisition. Sex was entered as a between-subject factor and block (1 vs. 2 vs. 3 vs. 4) was the within-subjects factor. Primary caregiver's marital status and prenatal cigarette exposure were included as covariates. Each of the three measures of prenatal alcohol exposure (AA/day, AA/occasion, and Frequency) was entered as a covariate in separate analyses. For AA/day, neither the main effect [$F(1, 79) = 1.21$; n.s.] nor the interaction with block were significant [$F(3, 237) = 1.37$; n.s.]. Similarly, for AA/occasion and frequency, neither the main effects ([$F(1, 79) = 1.38$; n.s.] and [$F(1, 79) = 1.50$; n.s.], respectively), nor the interactions with block were significant ([$F(3, 237) = 1.45$; n.s.] and [$F(3, 237) = 0.65$; n.s.], respectively).

Multiple regression analyses were performed to determine the relation between prenatal alcohol exposure and performance during the probe trial. AA per day and frequency of drinking were not related to prenatal alcohol exposure. AA/occasion was associated with increased time spent in the platform quadrant after controlling for sex, mother's age at delivery, and prenatal marijuana exposure (Table 4).

None of the three measures of prenatal alcohol exposure was related to mean trial time during cued navigation (Table 5).

3.4.3 CT-CS cohort

As described above, repeated measures ANCOVAs were conducted to determine whether prenatal alcohol exposure is associated with performance during acquisition. Sex was entered as a between-subject factor and block (1 vs. 2-3 vs. 4-5) was the within-subjects factor. Mother's age at delivery and age at testing were included as covariates. Each of the three measures of prenatal alcohol exposure (AA/day, AA/occasion, and Frequency) was entered as a covariate in separate analyses. For AA/day, the main effect [$F(1, 57) = 0.06$; n.s.] was not significant, but the interaction

with block approached significance [$F(2, 114) = 2.38$; $p = .097$]. No main effect [$F(1, 57) = 0.10$; n.s.] nor interaction with block [$F(2, 114) = 2.07$; n.s.] was detected for frequency of drinking. For AA/occasion, a significant interaction with block was detected [$F(2, 114) = 3.06$; $p < .05$]. The main effect of AA/occasion was not significant [$F(1, 57) = 0.58$; n.s.].

To further explore the interaction between AA/occasion and block, AA/occasion was grouped into binge exposed (AA/occasion ≥ 2) vs. Abstainers/Non-binge drinkers (AA/occasion < 2). Repeated-measures ANCOVA was performed with block as the within-subjects factor, and sex and the dichotomized AA/occasion as the between-subjects factors. Mother's age at delivery and age at testing were included as covariates. A significant AA/occasion group by block interaction was detected [$F(2, 112) = 3.23$; $p < .05$]. Figure 18 presents the mean trial time by block for the binge-exposed and non binge-exposed/abstainer groups. *T*-tests revealed that the binge-exposed children had significantly longer trial times than the non-binge exposed/abstainer group in blocks 2-3 ($t(60) = 2.06$; $p < .05$) and blocks 4-5 ($t(60) = 2.79$; $p < .01$). As expected a main effect of sex was also detected [$F(1, 56) = 9.76$; $p < .01$] in which girls had significantly longer trial times than boys. Figure 19 presents the mean trial time across each block for boys (A) and girls (B) separately. There were no significant AA/occasion group differences among girls, however binge-exposed boys performed more poorly than non-binge exposed boys in blocks 2-3 and 4-5.

Multiple regression analyses were performed to determine the relation between prenatal alcohol exposure and performance during the probe trial (Table 4). All three measures of prenatal alcohol exposure were associated with poorer performance during the probe trial, however after controlling for sex and age at testing, only AA/occasion and frequency remained significant.

None of the three measures of prenatal alcohol exposure was related to mean trial time during cued navigation (Table 5).

3.4.4 Detroit Longitudinal cohort

Repeated measures ANCOVAs were conducted to determine whether prenatal alcohol exposure is associated with performance during acquisition. Sex was entered as a between-subject factor and block (1 vs. 2-3 vs. 4-5) was the within-subjects factor. Age at testing, participant's current alcohol use (AA/day) and smoking, and prenatal cocaine exposure were included as covariates. Each of the three measures of prenatal alcohol exposure (AA/day, AA/occasion, and Frequency) was entered as a covariate in separate analyses. For AA/day, neither the main effect [$F(1, 97) = 1.32$; n.s.] nor the interaction with block were significant [$F(2, 194) = 1.13$; n.s.]. Similarly, for AA/occasion and frequency, neither the main effects ([$F(1, 97) = 0.61$; n.s.] and [$F(1, 97) = 2.40$; n.s.], respectively), nor the interactions with block were significant ([$F(2, 194) = 0.76$; n.s.] and [$F(2, 194) = 1.20$; n.s.], respectively).

Multiple regression analyses were performed to determine the relation between prenatal alcohol exposure and performance during the probe trial. AA per day, AA/occasion and frequency of drinking were not related to prenatal alcohol exposure (Table 4).

None of the three measures of prenatal alcohol exposure were related to mean trial time during cued navigation (Table 5).

3.5 Relation of FASD and prenatal alcohol exposure to hippocampal volume and virtual water maze performance

3.5.1 CT-L cohort

Figure 20 presents mean hippocampal volume by FASD diagnostic group, both before and after controlling for intracranial volume. There was a significant effect of FASD diagnostic group for both the left [$F(2, 128) = 3.47$; $p < .05$] (Fig. 20A) and right [$F(2, 128) = 8.40$; $p < .001$] (Fig. 20B) hippocampus. The FAS/PFAS group had significantly smaller left and right hippocampal volumes than both the HE and control groups (all ps $< .01$). After controlling for intracranial volume, the effects on the left [$F(2, 127) = 3.12$; $p < .05$], and right [$F(2, 127) = 7.37$; $p < .001$] hippocampal volumes remained significant. AAD was marginally associated with reduced right hippocampal volume, but after controlling for intracranial volume, this effect was no

longer significant (Table 6). Frequency of drinking during pregnancy was associated with smaller left and right hippocampal volumes, but after controlling for intracranial volume, only the right hippocampus approached statistical significance.

On the CT-L CG-1, trial time during block 3 was marginally related to right hippocampal volume (Table 7) but was no longer significant after control for intracranial volume. On CT-L CG-2, trial times during blocks 2 and 3 were marginally related to left hippocampal volume after, even after controlling for intracranial volume.

Analyses to examine whether hippocampal volume mediates the relation between FASD diagnostic group or prenatal alcohol exposure to VWM performance were not performed since a statistically significant relation between FASD diagnostic group/prenatal alcohol and VWM performance was not found (see chapters 5.3 and 5.4).

3.5.2 CT-CS cohort

There was a significant effect of FASD diagnostic group for both the left [$F(2, 47) = 3.46$; $p < .05$] (Fig. 21A) and right [$F(2, 47) = 4.53$; $p < .05$] (Fig. 21B) hippocampus. The FAS/PFAS group had significantly smaller left and right hippocampal volumes than both the control group (all ps $< .05$). The HE had smaller right hippocampal volume than the control group ($p < .05$). After controlling for intracranial volume, the effect on the left hippocampus was marginally significant [$F(2, 46) = 2.92$; $p = .064$]. The effect on the right hippocampus [$F(2, 46) = 3.63$; $p < .05$] remained significant after controlling for intracranial volume. For both the left and right, after controlling for intracranial volume, only the HE group significantly differed from controls (Fig. 21). All three measures of prenatal alcohol exposure were related to smaller left and right hippocampal volumes (Table 6). After controlling for intracranial volume, only the hippocampus was related to prenatal alcohol exposure.

Longer mean trial times during blocks 2-3 and 4-5 were associated with smaller left and right hippocampal volumes (Table 8). The relation between mean trial time during block 1 and left

and right hippocampal volume was marginally significant. After control for intracranial volume, VWM performance was no longer related to hippocampal volume.

Mediation analyses were performed to determine if hippocampal volume mediates the relation between FASD diagnostic group and prenatal alcohol exposure to VWM performance. Mean trial time in blocks 4-5 during acquisition was used as the dependent variable since it reflects place learning performance at the end of the acquisition phase, and it was the most strongly related to both hippocampal volume and FASD diagnostic group/prenatal alcohol exposure (see chapters 5.3.3 and 5.4.3).

Figure 22 is a path model showing the mediation by right hippocampal volume of the relation between FASD diagnostic group and VWM performance controlling for sex. The HE and FAS/PFAS independent variables were indicator coded using the controls as the reference group. The model accounted for 20.5% of the variance in the dependent variable [$F(4, 45) = 4.15$; $p < .01$]. Both of the indirect effects, i.e., the paths from FAS/PFAS and HE through right hippocampal volume to VWM were significant; HE indirect pathway effect = 1.49 (95% confidence interval = 0.04, 4.85), FAS/PFAS indirect pathway effect = 2.66 (95% confidence interval = 0.34, 6.69). The same model was re-run using left hippocampal volume instead of right, however, the indirect effects were not significant, HE indirect pathway effect = 1.01 (95% CI = -0.14, 3.87), FAS/PFAS indirect pathway effect = 1.87 (95% CI = -0.27, 6.38). When intracranial volume was added to the model for right hippocampal volume as a covariate, the indirect effects were no longer significant, HE = 1.21 (95% CI = -0.41, 5.20), FAS/PFAS = 0.50 (95% CI = -0.41, 3.10). Since the addition of intracranial volume to the model eliminated the significance of the indirect effect through right hippocampal volume, it could be hypothesized that the effect of FASD diagnostic group on VWM performance is mediated by total brain volume reductions and not specific to the hippocampus. To test this hypothesis a second model testing intracranial volume as a mediator was performed (Figure 23). The direct effect of intracranial volume on VWM performance was not significant nor were the indirect effects through intracranial volume HE = 0.03 (95% CI = -1.25, 1.67), FAS/PFAS

= 2.49 (95% CI = -0.64, 8.43) indicating that intracranial volume is not a significant mediator of the relation between FASD diagnostic group and VWM performance.

Figure 24 presents the mediation of right hippocampal volume on the relation between prenatal alcohol binge exposure and VWM performance controlling for sex. The prenatal alcohol binge exposure independent variable is the same dichotomous variable described in chapter 5.4.3. The model accounted for 15.2% of the variance in the dependent variable [$F(3, 46) = 4.15$; $p < .05$]. The indirect path was significant; product of regression coefficients = 1.78 (95% confidence interval = 0.21, 4.73). The same model was re-run using left hippocampal volume instead of right, but the indirect path was not significant, effect = 1.31 (95% CI = -0.02, 4.40). When intracranial volume was added to the model as a covariate, the indirect path through right hippocampal volume was no longer significant, effect = 0.49 (95% CI = -0.31, 2.80). A second model testing intracranial volume as a mediator was performed (Figure 25). The indirect effect through intracranial volume was not significant, effect = 1.32 (95% CI = -0.05, 4.62) indicating that intracranial volume was not a significant mediator of the relation between prenatal alcohol binge exposure and VWM performance.

3.6 Relation of prenatal alcohol exposure to testosterone and virtual water maze performance

Previous data from this cohort has shown that prenatal alcohol exposure is associated with increased salivary testosterone levels at 14 years of age (Carter et al., 2014). This same relation was confirmed in this subset ($N = 37$) of those participants from that larger study who had both salivary testosterone data from the 14-year visits and VWM data from the 18-year follow-up. AA/day (Pearson's $r = 0.30$; $p < .10$) and frequency of drinking (Pearson's $r = 0.32$; $p < .05$) during pregnancy were related to elevated salivary testosterone. AA/occasion was not related to testosterone (Pearson's $r = 0.03$). Table 9 displays the correlations between salivary testosterone at 14 years and VWM performance at 18-years. Testosterone levels were not related to VWM performance.

CHAPTER 4 DISCUSSION

The purpose of this study was to assess the effects of prenatal alcohol exposure on VWM performance, a test of place learning that is hippocampal dependent. The main hypotheses of this project were that prenatal alcohol exposure and/or FASD diagnosis is related to poorer performance on the VWM/CG Arena. Although the prenatal alcohol effects were not seen in the moderately exposed Detroit longitudinal and in the CT-L cohorts, this hypothesis was supported when place learning was assessed using the VWM test in the very heavily exposed CT-CS cohort. This project was the first to examine FASD-related deficits on VWM performance in both sexes and among non-syndromal, heavily exposed individuals. The effects found in the CT-CS cohort for both sexes and in the non-syndromal groups were not mediated by impairments to global intellectual function characterized by heavy prenatal alcohol exposure. This study is also the first to show that the amount of prenatal alcohol consumed per occasion is associated with deficits in place learning and the first to show that fetal alcohol-related volume reductions to the hippocampus mediate the relation between prenatal alcohol exposure and VWM performance. The analyses also showed that testosterone levels did not impact on VWM performance, and, therefore, cannot be responsible for any alcohol-related effects on place learning.

4.1 Effect of FASD diagnostic group on VWM performance

In the very heavily exposed CT-CS cohort, this study found that VWM performance was impaired in both boys and girls diagnosed with FAS or PFAS and in boys who were non-syndromal, but heavily exposed. These findings are in agreement with Hamilton et al.'s (2003) findings that boys with FAS were impaired on the VWM. Additionally, the findings here address several of the limitations in the Hamilton et al. (2003) study. First, by assessing girls as well as boys, this study was able to show that FASD-related deficits in place learning are detectable in both sexes using the VWM. Second, in addition to the finding of impairment in the FAS/PFAS group, this study was able to detect effects in non-syndromal, heavily exposed boys demonstrating that impairments in place learning are also seen in those lacking the physical

markers of FASD. Third, the effects detected on the VWM persisted after controlling for IQ, demonstrating that fetal alcohol-related deficits in place learning occur over and above deficits in global functioning, even among the most severely affected FAS/PFAS groups.

These findings of fetal alcohol impairment on the VWM were limited to the CT-CS cohort and not seen in the CT-L cohort. A likely source for the discrepancy in findings between the cohorts is the degree of prenatal alcohol exposure. Although both cohorts were comparable in terms of socioeconomic status, child's age, and IQ, the mothers who drank in the CT-CS cohort consumed more than three times as much alcohol per day, 70% more alcohol per occasion, on average, and drank twice as often as the mothers in the CT-L cohort (Tables 1-2). It should be noted that alcohol was ascertained retrospectively in the CT-CS cohort, which often yields higher reports of pregnancy alcohol consumption than when ascertained during the pregnancy (Ernhart, Morrow-Tlucak, Sokol, & Martier, 1988; Hannigan et al., 2010; Jacobson et al., 2002). However, even when comparing retrospective reports of pregnancy drinking from the mothers in the CT-L cohort ascertained at 5 years postpartum, their retrospectively-reported alcohol use during pregnancy (*M*s = 1.2 oz AA/day; oz AA/occasion = 3.9; Frequency = 1.9 days/week) was still not nearly as high as in the CT-CS cohort. These data suggest that deficits on the VWM are seen only at the highest levels of prenatal alcohol exposure. Further work is needed to confirm this conclusion, as the only comparable study to date did not report quantitative measures of alcohol consumption (Hamilton et al., 2003).

Another possible reason for this discrepancy could be the differences in total number of trials administered during acquisition between the two cohorts. The CT-L cohorts were administered 16 acquisition trials, while the CT-CS cohort was administered 20 acquisition trials. The four extra trials administered to the CT-CS cohort could have allowed more learning to occur in controls, thus allowing a lower floor and greater between-group differences in the CT-CS cohort. If this was the case, it would be expected that controls in the CT-CS cohort would have lower mean trial times at the end of acquisition compared to controls in the CT-L cohort. However,

the control group in the CT-L cohort had lower mean trial times at the end of acquisition (*M* = 28.4) than the controls in the CT-CS cohort (*M* = 31.8), suggesting that the extra trials did not facilitate performance. Another difference between the two tasks was the number of room cues used. The VWM which was administered to the CT-CS and Detroit cohorts used four distal room cues, while the CG Arena administered to the CT-L cohort used eight total room cues. While it is not clear what the effect of number of cues has in humans, in rodent studies increasing the number of cues used does not facilitate performance (Fenton, Arolfo, Nerad, & Bures, 1994).

4.2 Effect of prenatal alcohol exposure on VWM performance

This was the first study to examine the effects of a continuous measure of prenatal alcohol exposure on VWM performance. In the CT-CS cohort, dose per occasion was related to poorer acquisition, and on the probe trial both dose per occasion and frequency of drinking were related to less time spent searching the platform quadrant. Similar to the findings relating FASD diagnosis to VWM performance, effects were detected only in the most heavily-exposed CT-CS cohort, and not in the somewhat less heavily exposed CT-L cohort, nor the moderately exposed Detroit cohort. The conclusion that these effects are seen only in very heavily exposed children is supported by the fact that the Detroit cohort was administered the exact same version of the VWM as the CT-CS cohort.

The finding that AA/occasion, rather than AA/day was most sensitive in detecting effects on the VWM suggests that the pattern of prenatal alcohol exposure rather than total amount of exposure may be particularly important in producing place learning deficits. AA/occasion is more indicative of concentrated alcohol use, such as binge drinking when peak blood alcohol concentrations reach high levels. These data also show impairments on the VWM are detected primarily in relation to a binge pattern of exposure. This finding is in agreement with the rodent literature which has found concentrated binge-like exposure produce severe Morris water maze deficits, while chronic, lower-level exposure only produces minimal effects (Goodlett et al., 1987; Kelly et al., 1988). Furthermore, place learning is dependent on the hippocampus in both rodents

(Morris et al., 1982; Moser et al., 1993; 1995; Pearce et al., 1998) and humans (Astur et al., 2002; Goodrich-Hunsaker et al., 2010), a region which is particularly vulnerable to concentrated binge-like alcohol exposure (Bonthius & West, 1990; Green et al., 1992; Livy et al., 2003).

4.3 Hippocampal volume, virtual water maze performance and FASD

Previous studies in rodents (Morris et al., 1982; Moser et al., 1993; 1995; Riedel et al., 1999) and humans (Astur et al., 2002; Bohbot et al., 2007; Brown et al., 2014; Goodrich-Hunsaker et al., 2010; Hsu et al., 2000; Moffat et al., 2006; Paslow et al., 2004) have demonstrated that the hippocampus is critical for the encoding and retrieval of spatial relations needed for successful navigation based on distal cues. In this study, hippocampal volume was correlated with poorer performance during acquisition in the virtual water maze, although this effect did not persist after controlling for total intracranial volume. Two prior studies using voxel based morphometry found that hippocampal volume was associated with the use of mapping strategies employed during navigation even after correcting for brain size (Bohbot et al., 2007; Brown et al., 2014). Together, these findings lend further support to the role of the hippocampus in the encoding and retrieval of spatial information for navigation. Voxel-based morphometry may provide more accurate measurement of hippocampal volume, when compared to the FreeSurfer method used in this study.

In this study, both FASD diagnostic group and degree of prenatal alcohol exposure were related to hippocampal volumes reductions even after controlling for differences in intracranial volume. In the CT-L cohort, both left and right hippocampal volume was reduced in the FAS/PFAS group even after control for intracranial volume. In the CT-CS cohort, the reductions in left and right hippocampal volumes remained significant after controlling for intracranial volume only for the HE group relative to the controls. These findings are consistent with a number of studies showing hippocampal reductions in relation to prenatal alcohol exposure with (Archibald et al., 2001; Nardelli et al., 2011; Willoughby et al., 2008) and without (Astley et al., 2009; Coles et al.,

2010; Joseph et al., 2014; Riikonen et al., 2005; Roussotte et al., 2011) accounting for total brain size.

Although significant hippocampal volume reductions were detected only in the FAS/PFAS group in the CT-L cohort, in the CT-CS cohort they were also detected in the non-syndromal HE group. The finding of smaller hippocampal volume in the HE group only in the latter cohort is consistent with the higher levels of prenatal alcohol exposure in that cohort. These data thus show that hippocampal volume reductions occur even among those with very heavy prenatal alcohol exposure who lack the overt physical features of FASD. Two prior studies also detected hippocampal volume reductions in participants with FASD lacking the physical dysmorphia required for a diagnosis of FAS or PFAS, compared to non-exposed controls (Astley et al., 2009; Coles et al., 2010). However, two other studies making this comparison did not detect significant hippocampal volume differences among the non-dysmorphic exposed group (Archibald et al., 2001; Nardelli et al., 2011), suggesting possibly lower levels of exposure, which was not measured in those cohorts.

This is the first study to report direct associations between amount of prenatal alcohol exposure and hippocampal volume. Hippocampal volume was negatively associated with degree of prenatal alcohol exposure in both the CT-L and CT-CS cohorts and, after control for intracranial volume, this effect persisted in the CT-CS cohort. All prior studies documenting hippocampal volume reductions related to prenatal alcohol exposure have compared groups based on exposed vs. non-exposed (Joseph et al., 2014; Nardelli et al., 2011; Riikonen et al., 2005; Roussotte et al., 2011; Willoughby et al., 2008) or on qualitative FASD diagnostic groups (Archibald et al., 2001; Astley et al., 2009; Coles et al., 2010). Taken together with the findings comparing the FASD diagnostic groups, these data demonstrate that hippocampal volume is related both to the degree of prenatal alcohol exposure and markers of prenatal alcohol effect, (i.e. FASD diagnosis).

The hypothesis that hippocampal volume would mediate the relation between FASD and VWM performance was supported. In the CT-CS cohort, right hippocampal volume was a

significant mediator when either FASD diagnostic group or binge-exposure group was used as the independent variable. As indicated earlier, when total intracranial volume was used as a covariate in the mediation models, mediation by hippocampal volume was no longer significant, probably due to the high correlations between hippocampal and total volume. However, given that total intracranial volume itself did not mediate the relation between FASD diagnostic group of binge exposure and VWM performance, it seems reasonable to infer that the observed mediation is due specifically to the smaller hippocampal volume. Left hippocampal volume was not a significant mediator. Both left and right hippocampal volume predicted VWM performance, and volume reductions were detected in both hippocampi in relation to prenatal alcohol exposure. Functional MRI studies of navigation have consistently found bilateral activation of the hippocampus (Hsu et al., 2000; Maguire et al., 2008; Parslow et al., 2004). A more recent fMRI study demonstrated that activation in the right hippocampus predicted the use of allocentric navigation, that is, navigation based on the spatial configuration of environmental cues, while activation in the left hippocampus predicted the use of sequential egocentric representations, such as learning the sequence of direction changes needed to navigate to a goal (Igloi, Doeller, Berthoz, Rondi-Reig, & Burgess, 2010). The finding that right hippocampal volume was more important than left in mediating the effect of prenatal alcohol exposure on the VWM in this study is not surprising given that the an allocentric navigation strategy is most efficient for learning the location of the hidden platform. Similar to the findings reported here, Coles et al. (2010) found that the right hippocampus volume primarily mediated the relation between the number of alcohol-related dysmorphic features and verbal and nonverbal recall. Taken together with the findings from this study, prenatal alcohol-related insult to the hippocampus appears to impair not only place learning, but also verbal and non-verbal learning, suggesting a general deficit in the encoding of information mediated by the hippocampus.

4.4 Testosterone, FASD, and virtual water maze

As expected, prenatal alcohol exposure was related to higher salivary testosterone levels in this subset of participants from the Carter et al. (2014) study. Testosterone levels were not related to VWM performance, a finding consistent with those of Burkitt et al. (2007) who found no differences in performance based on low or high levels of endogenous testosterone in males. However, it should be noted that the lack of findings reported in this study could be due to the lack of power from the small number of subjects (*N* = 37) who had both testosterone and VWM. Further studies with a larger number of participants are needed.

4.5 Limitations

There are some limitations to the present study. First, two of the cohorts were selected from a very impoverished community in Cape Town, South Africa, while the third cohort from Detroit was selected from an economically-disadvantaged inner city populations raising the question about how well the results reported here will generalize to other populations. Second, all three cohorts were selected on the basis of exposure, and not effect. It could be argued that, the full range of clinical outcomes from prenatal alcohol exposure may, therefore, be somewhat under-represented in these samples. However, a broad range of clinical outcomes were detected in these cohorts. Moreover, this design is beneficial in that it allows quantitative exposure histories to be well documented, and allows for the study of factors that determine effect given similar exposure histories. Lastly, there was evidence that hippocampal volume mediates the relation of FASD/prenatal alcohol exposure and VWM performance. Regional brain volume can be considered a relatively crude measure of teratological insult in that it does not indicate whether the deficit in function is localized to the region in question, the neurocognitive functional networks it participates in, or both. Further research using functional MRI could help in determining how prenatal alcohol exposure affects the network of brain regions involved in spatial navigation.

4.6 Conclusions

This study has demonstrated that at very heavy levels of prenatal alcohol exposure both FASD diagnosis and degree of exposure are related to impairment on the VWM in both boys and

girls. In terms of FASD diagnosis, both dysmorphic and non-dysmorphic exposed individuals are impaired, a finding that confirms and extends the findings of Hamilton et al. (2003). This was the first study to show that degree of prenatal alcohol exposure is associated with impairment on the VWM and the first to show that this effect is significant even after controlling for environmental influences, prenatal exposure to other drugs, and overall intellectual function. These findings held only for the cohort with the heaviest level of prenatal alcohol exposure, suggesting that the VWM is not sensitive to effects from low-to-moderate levels of prenatal alcohol exposure. The hippocampus was shown to be vulnerable to prenatal alcohol exposure, and there was evidence that volume reductions in this region mediate the relation between prenatal alcohol exposure and VWM performance. These data suggest that prenatal alcohol exposure leads to damage to the hippocampus, which impairs the ability to encode the spatial information necessary for successful location of the hidden platform on the VWM. These findings add to the growing body of evidence suggesting that hippocampal damage plays an important role in the teratogenic effects of this exposure.

APPENDIX A TABLES

Table 1. Cape Town Longitudinal Cohort sample characteristics

	FAS (*N* = 8)	PFAS (*N* = 4)	HE (*N* = 34)	Control (*N* = 38)	*F* or χ^2
Maternal characteristics					
Mother's age at delivery	29.4 (4.5)	25.6 (4.7)	24.9 (4.6)	25.7 (5.8)	1.56
Primary caregiver's education (yr)	8.3 (3.0)	8.0 (2.6)	9.7 (2.3)	9.9 (2.0)	1.69
Primary caregiver's marital status (% married)	0.0	50.0	38.2	60.5	10.90*
Prenatal exposure					
Cigarettes per day	3.1 (3.5)	9.4 (9.6)	5.9 (5.2)	4.0 (5.8)	1.78
Alcohol					
oz AA/day	0.9 (0.6)	0.8 (0.8)	0.7 (0.8)	0.003 (0.002)	12.43**
oz AA/occasion	4.8 (2.5)	3.0 (1.4)	3.5 (3.3)	0.02 (0.1)	19.39**
Frequency (days/week)	1.7 (0.7)	3.0 (1.6)	2.0 (1.7)	0.02 (0.1)	22.43**
Child characteristics					
Age at testing	10.7 (0.7)	10.0 (0.7)	10.9 (0.6)	10.7 (0.5)	3.00*
Sex (% male)	75.0	50.0	52.9	47.4	2.03
IQ	62.8 (14.6)	72.0 (6.8)	78.4 (15.8)	75.6 (14.6)	2.44†

Values are Mean (SD) or %. AA = Absolute alcohol.

†$p < .10$ *$p < .05$ **$p < .001$

Table 2. Cape Town Cross-Sectional Cohort sample characteristics

	FAS (*N* = 7)	PFAS (*N* = 4)	HE (*N* = 26)	Control (*N* = 25)	*F* or χ^2
Maternal characteristics					
Mother's age at delivery	28.7 (5.9)	23.9 (4.1)	24.6 (5.5)	24.0 (5.8)	1.33
Primary caregiver's education (yr)	6.4 (3.5)	8.0 (2.1)	6.9 (2.3)	8.5 (2.0)	2.82*
Primary caregiver's marital status (% married)	28.6	50.0	50.0	64.0	3.00
Prenatal exposure					
Cigarettes per day	7.7 (7.7)	7.3 (2.5)	11.1 (8.6)	5.6 (7.1)	2.22†
Alcohol					
oz AA/day	3.1 (2.9)	2.8 (2.2)	2.3 (2.4)	0.002 (0.01)	9.31**
oz AA/occasion	6.3 (1.8)	7.7 (5.0)	5.9 (5.3)	0.1 (0.3)	13.65**
Frequency (days/week)	3.1 (1.8)	2.5 (0.6)	2.7 (1.6)	0.01 (0.1)	24.97**
Child characteristics					
Age at testing	10.1 (1.7)	9.4 (0.4)	10.7 (1.2)	10.2 (1.2)	1.66
Sex (% male)	42.9	25.0	46.2	48.0	0.76
IQ	58.2 (7.5)	71.2 (10.7)	66.6 (10.0)	76.0 (11.0)	6.88**

Values are Mean (SD) or %. AA = Absolute alcohol.

†$p < .10$ *$p < .05$ **$p < .001$

Table 3. Detroit Longitudinal Cohort sample characteristics (*N* = 104)

	N	*M* or %	SD	Range
Maternal characteristics				
Mother's age at delivery	104	26.6	6.0	15.1 - 39.1
Prenatal exposure[a]				
Cigarettes per day	62	14.0	10.5	1.0 - 41.0
Marijuana use (days/month)	35	3.4	3.2	0.3 - 15.1
Cocaine (days/month)	37	5.3	4.2	0.3 - 17.0
Alcohol				
oz AA/day	81	0.3	0.8	0.001- 5.0
oz AA/occasion	81	2.0	3.3	0.1 - 24.8
Frequency (days/week)	81	1.0	1.3	0.1 - 7.0
Adolescent characteristics				
Age at testing	104	19.4	0.6	18.3 - 21.0
Sex (male)	58	55.8		
IQ at age 14yr[b]	94	80.1	14.0	48.0 - 121.0
Current cigarettes/day[a]	16	5.3	6.4	0.3 - 20.0
Current alcohol use (oz AA/day)[a]	35	0.3	0.3	0.02 - 1.4
Current marijuana use (days/month)[a]	35	13.8	13.1	1.0 - 30.0

AA = Absolute alcohol.

[a]Users only.

[b]Missing for 10 cases who did not participate in the 14-year follow-up.

Table 4. Effects of prenatal alcohol exposure on probe trial performance in each cohort

		AA/day		AA/occasion		Frequency	
	N	*r*	*β*	*r*	*β*	*r*	*β*
Cape Town Longitudinal—CG 1[a]	47	-.16	-.15	-.03	-.03	-.21†	-.21
Cape Town Longitudinal—CG 2[b]	84	.03	.17	.14	.24*	.03	.13
Cape Town Cross-sectional[c]	62	-.20†	-.18	-.25*	-.24†	-.22*	-.21†
Detroit Longitudinal[d]	104	.07	.04	.06	.04	.01	-.04

Values are Pearson's *r* and standardized regression coefficients (*β).*

[a]Controlling for sex. [b]Controlling for sex, mother's age at delivery, and prenatal marijuana exposure. [c]Controlling for sex and age at testing. [d]Controlling for sex and mother's age at delivery.

†$p < .10$ *$p < .05$

Table 5. Effects of prenatal alcohol exposure on trial time during cued navigation in each cohort

		AA/day		AA/occasion		Frequency	
	N	*r*	*β*	*r*	*β*	*r*	*β*
Cape Town Longitudinal—CG 1[a]	46	.10	.03	.01	-.09	.11	.08
Cape Town Longitudinal—CG 2[b]	84	-.03	-.07	-.07	-.10	-.03	-.08
Cape Town Cross-sectional[c]	62	.16	-.01	.13	-.01	.14	.01
Detroit Longitudinal[a]	104	-.07	-.03	.02	.05	-.13	-.08

Values are Pearson's *r* and standardized regression coefficients (*β*).

[a]Controlling for sex. [b]Controlling for sex and age at testing. [c]Controlling for sex, age at testing, marital status, and prenatal cigarette exposure.

Table 6. Effects of prenatal alcohol exposure on hippocampal volume in the Cape Town cohorts

	AA/day		AA/occasion		Frequency	
	r	$r_{partial}$	*r*	$r_{partial}$	*r*	$r_{partial}$
Cape Town Longitudinal (*N* = 121)						
R Hippocampus	-.12†	-.09	-.05	-.05	-.18*	-.14†
L Hippocampus	-.11	-.07	-.05	-.04	-.16*	-.11
Cape Town Cross-sectional (*N* = 50)						
R Hippocampus	-.35*	-.31*	-.33*	-.27†	-.30*	-.31*
L Hippocampus	-.29*	-.22	-.30*	-.23	-.25†	-.23

Values are Pearson's *r* and partial *r* controlling for intracranial volume.

†$p < .10$ *$p < .05$

Table 7. The relation of virtual water maze performance to hippocampal volume in the Cape Town Longitudinal cohort

	Hippocampal volume			
	Left		Right	
	r	$r_{partial}$	r	$r_{partial}$
CG 1—120 sec/trial (N = 42)				
Acquisition (mean trial time)				
Block 1	-.16	.05	-.08	.11
Block 2	-.05	.18	-.05	.13
Block 3	-.20	-.03	-.26†	-.10
Block 4	-.05	.13	-.17	-.07
Probe trial (% time in platform quadrant)	.05	-.15	.07	-.11
CG 2—60-sec/trial (N = 79)				
Acquisition (mean trial time)				
Block 1	-.15	-.16	-.04	-.05
Block 2	-.17	-.20†	-.02	-.04
Block 3	-.20†	-.19†	-.04	-.02
Block 4	.03	.004	.03	.001
Probe trial (% time in platform quadrant)	-.05	-.07	.02	-.01

Values are Pearson's r and partial r controlling for intracranial volume.

†$p < .10$

Table 8. The relation of virtual water maze performance to hippocampal volume in the Cape Town Cross-Sectional cohort (N = 50)

	Hippocampal volume			
	Left		Right	
	r	$r_{partial}$	r	$r_{partial}$
Acquisition (mean trial time)				
Block 1	-.25†	.02	-.27†	-.01
Blocks 2-3	-.55***	-.16	-.48***	-.05
Blocks 4-5	-.35*	-.09	-.40**	-.18
Probe trial (% time in platform quadrant)	.08	-.06	.09	-.03

Values are Pearson's r and partial r controlling for intracranial volume.

$^{\dagger}p < .10$ $^{*}p < .05$ $^{**}p < .01$ $^{***}p < .001$

Table 9. Effects of salivary testosterone on virtual water maze performance

	Testosterone		
	Total (*N* = 37)	Boys (*N* = 20)	Girls (*N* = 17)
Acquisition (mean trial time)			
Block 1	.23	.14	-.35
Blocks 2-3	.06	.04	.15
Blocks 4-5	-.02	-.28	.24
Probe trial (% time in platform quadrant)	.03	.21	-.09

Values are Pearson's *r*.

APPENDIX B FIGURES

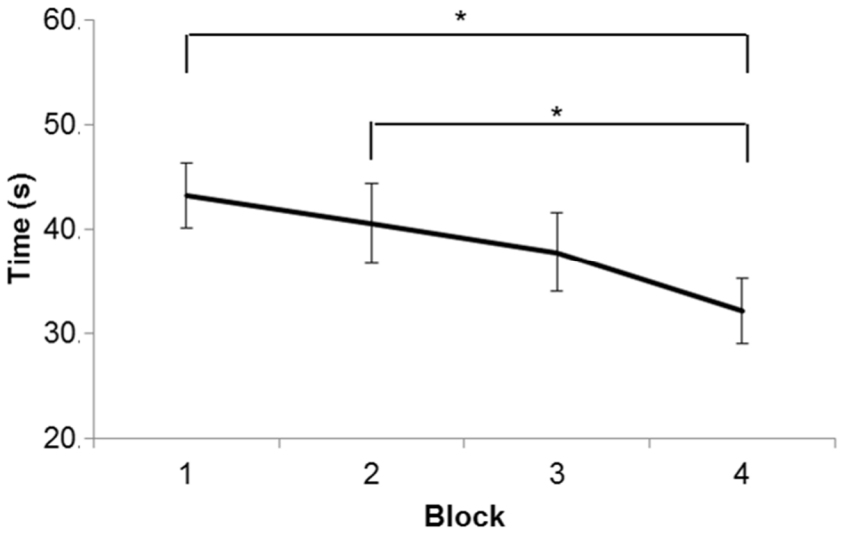

Figure 1. Mean (± S.E.) trial time to find the hidden platform during acquisition (hidden platform) in the 120-sec/trial version (CG-1) of the CG Arena water maze, CT-L cohort. Asterisks indicate significant difference from Block 1; *$p < .05$.

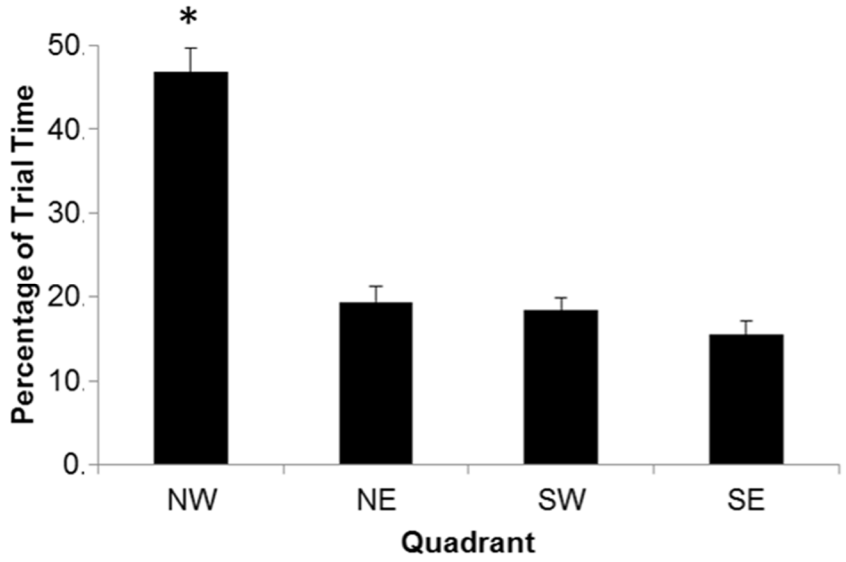

Figure 2. Mean (+ S.E.) percentage of time spent in each pool quadrant during the probe trial of the 120-sec/trial version (CG-1) of the CG Arena water maze water maze, CT-L cohort. During place learning the hidden platform was located in the NW quadrant. Participants spent the majority of time searching this quadrant. Asterisk indicates percentage of time in the NW quadrant was statistically higher than percentage spent in the other quadrants, all *p*s < .001.

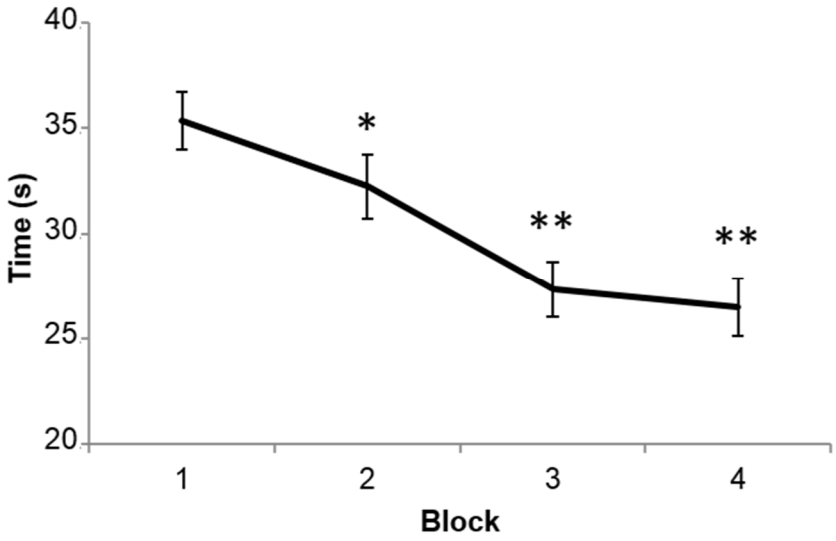

Figure 3. Mean (± S.E.) trial time to find the hidden platform during acquisition (hidden platform) in the 60-sec/trial version (CG-2) of the CG Arena water maze water maze, CT-L cohort. Asterisks indicate significant difference from Block 1; *$p < .05$, **$p < .001$.

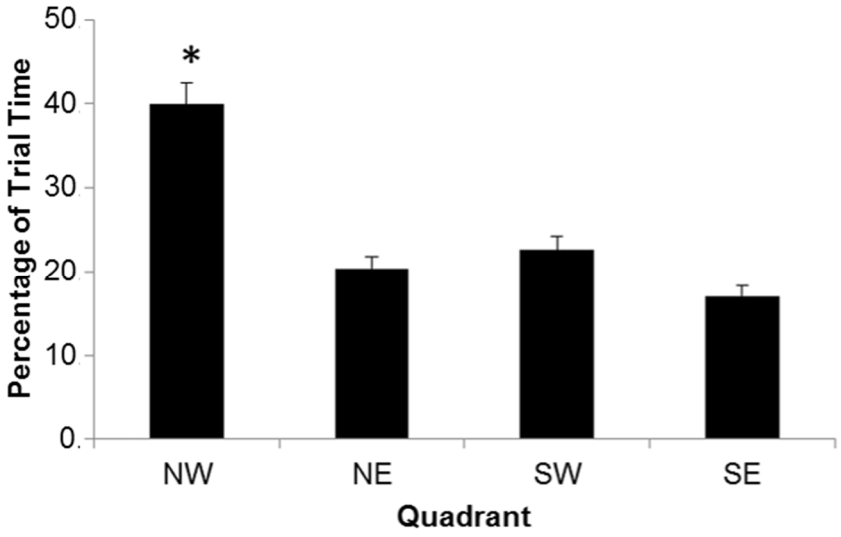

Figure 4. Mean (+ S.E.) percentage of time spent in each pool quadrant during the probe trial the 60-sec/trial version (CG-2) of the CG Arena water maze water maze, CT-L cohort. During place learning the hidden platform was located in the NW quadrant. Participants spent the majority of time searching this quadrant. Asterisk indicates percentage of time in the NW quadrant was statistically higher than percentage spent in the other quadrants, all *p*s < .001.

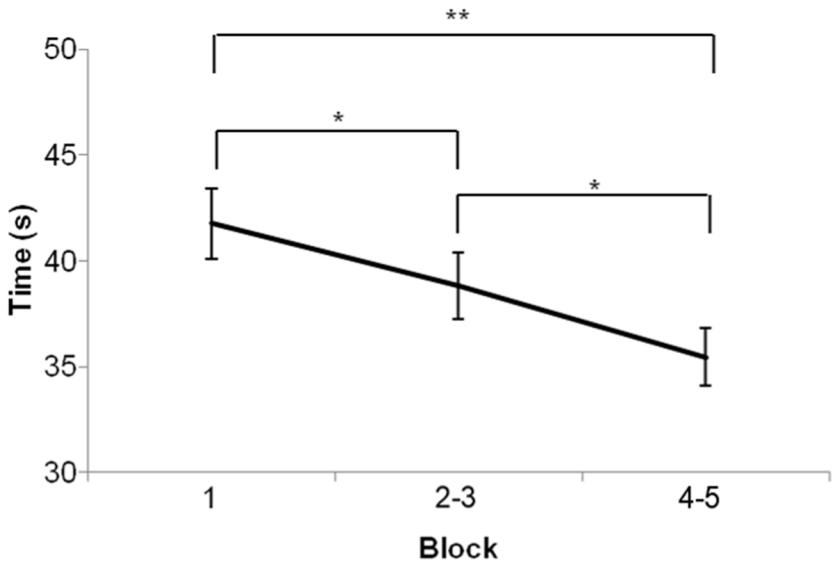

Figure 5. Mean (± S.E.) trial time to find the hidden platform during acquisition (hidden platform) in the CT-CS cohort. Asterisks indicate significant differences between blocks. *$p < .05$, **$p < .001$.

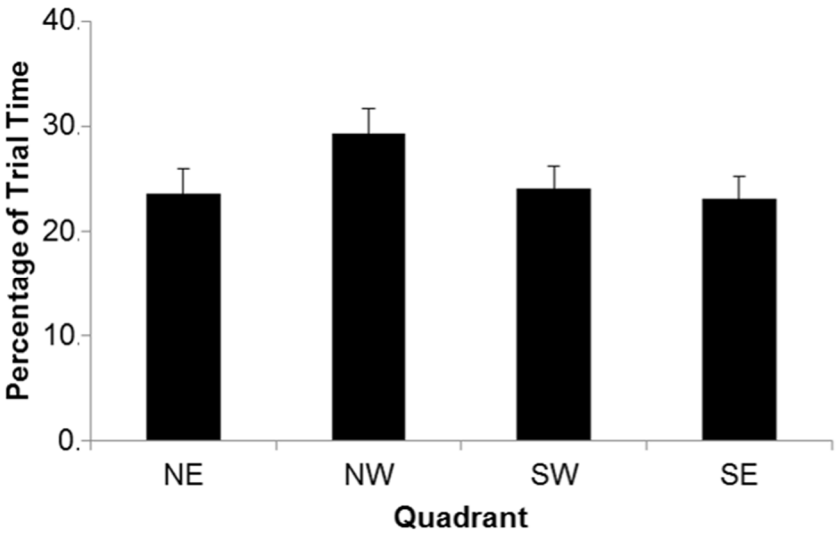

Figure 6. Mean (+ S.E.) percentage of time spent in each pool quadrant during the probe trial in the CT-CS cohort. During place learning the hidden platform was located in the NE quadrant. No differences were detected.

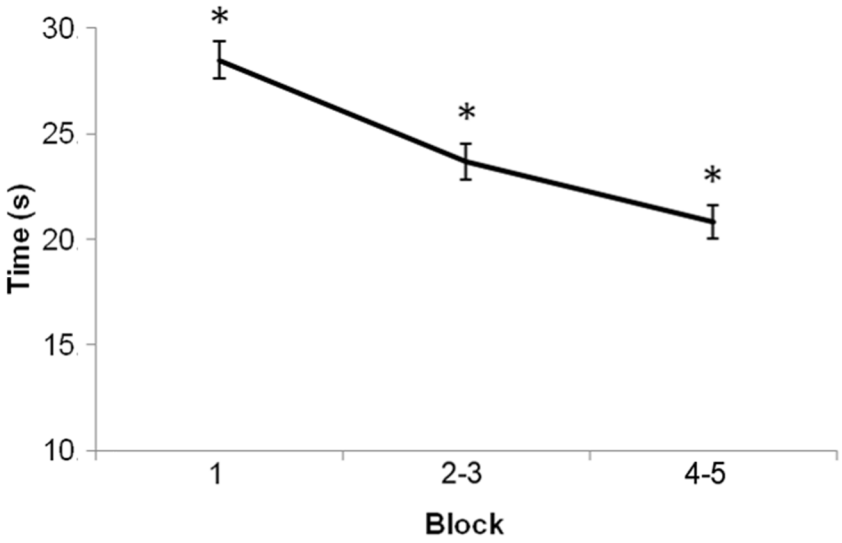

Figure 7. Mean (± S.E.) trial time to find the hidden platform during acquisition (hidden platform) in the Detroit cohort. All blocks were significantly different from each other. *$p < .001$.

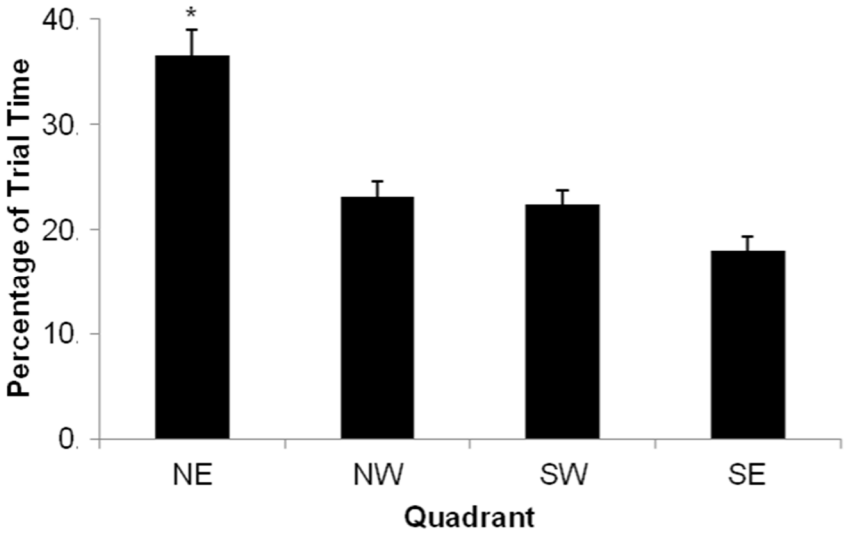

Figure 8. Mean (+ S.E.) percentage of time spent in each pool quadrant during the probe trial in the Detroit cohort. During place learning the hidden platform was located in the NE quadrant. Participants spent the majority of time searching this quadrant. Asterisk indicates percentage of time in the NE quadrant was statistically higher than percentage spent in the other quadrants, all *p*s < .001.

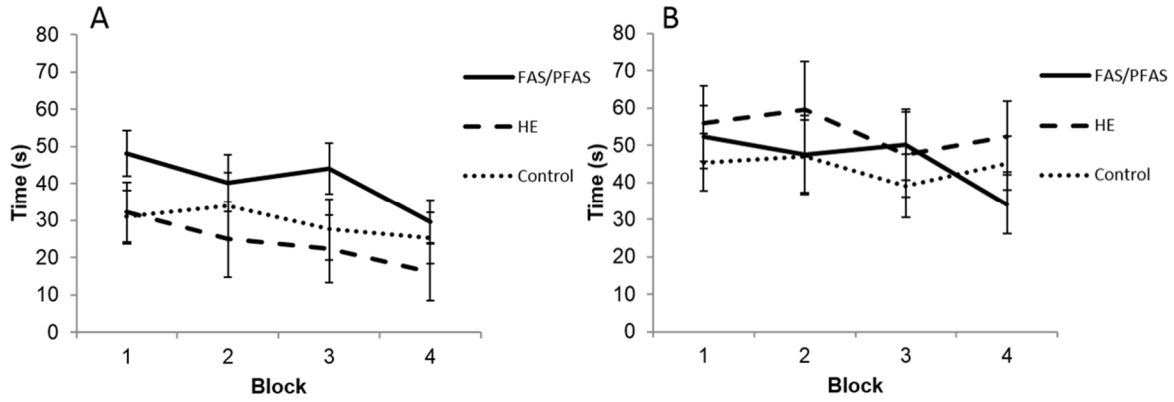

Figure 9. Mean (± S.E.) trial time to locate the hidden platform during acquisition by FASD diagnostic group for boys (A) and girls (B) in version 1 of the CG Arena water maze task in the CT-L cohort. Values are adjusted for covariates in the model; primary caregiver's marital status, and prenatal cigarette exposure.

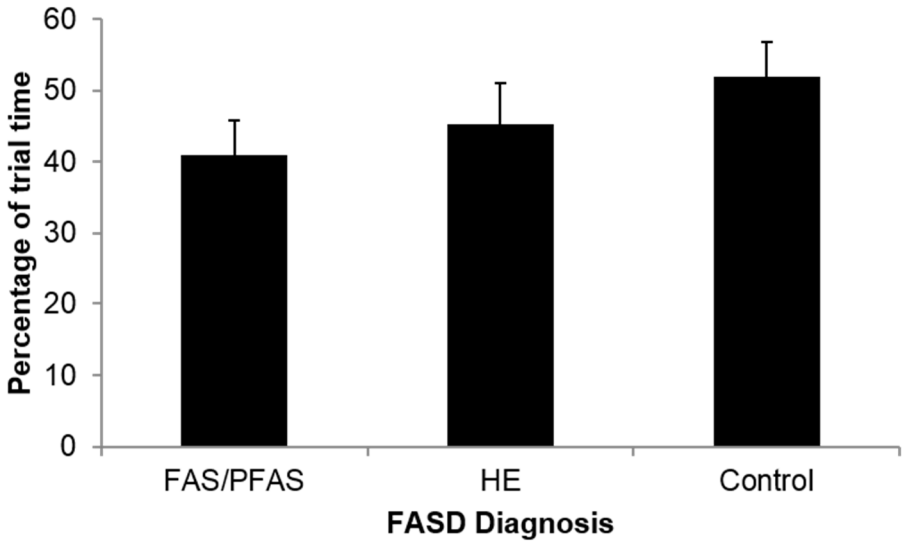

Figure 10. Mean (+ S.E.) percentage of time spent in the platform quadrant (NW) during the probe trial by FASD diagnostic group in version 1 of the CG Arena water maze task in the CT-L cohort. No significant differences were detected between diagnostic groups.

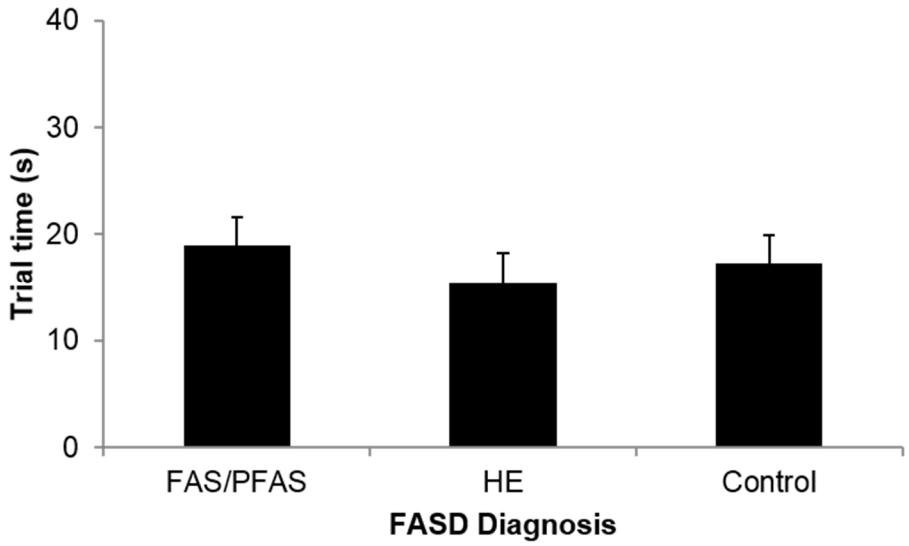

Figure 11. Mean (+ S.E.) trial time during cued navigation trials by FASD diagnostic group in version 1 of the CG Arena water maze task in the CT-L cohort. Values are adjusted for the covariate prenatal cigarette exposure. No significant differences were detected between diagnostic groups.

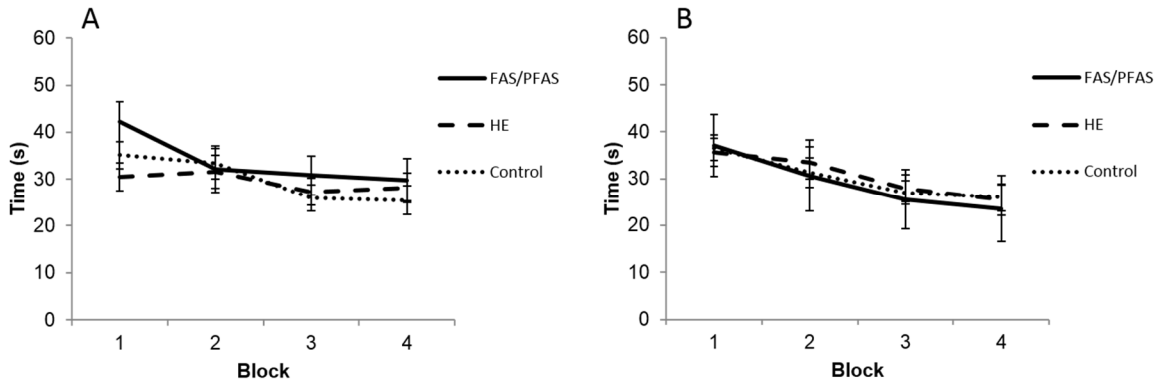

Figure 12. Mean (± S.E.) trial time to locate the hidden platform during acquisition by FASD diagnostic group for boys (A) and girls (B) in version 2 of the CG Arena water maze task in the CT-L cohort. Values are adjusted for covariates in the model; socioeconomic status, prenatal marijuana exposure, age at testing.

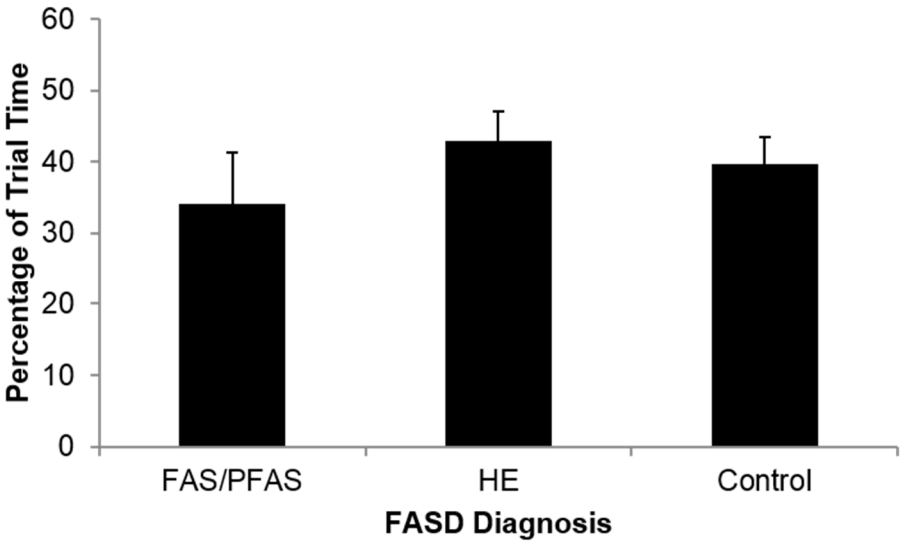

Figure 13. Mean (+ S.E.) percentage of time spent in the platform quadrant during the probe trial by FASD diagnostic group in version 2 of the CG Arena water maze task in the CT-L cohort. Values are adjusted for the covariates mother's age at delivery, prenatal marijuana exposure. No significant differences were detected between diagnostic groups.

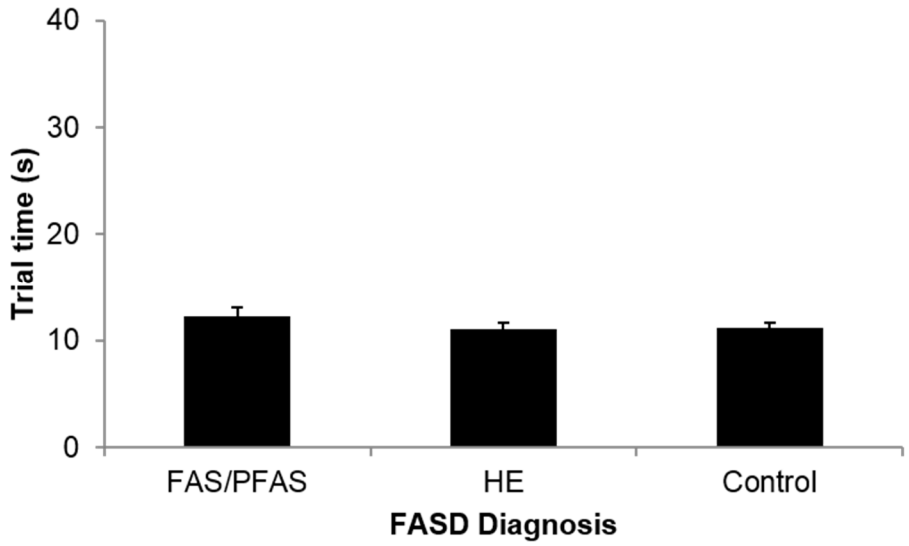

Figure 14. Mean (+ S.E.) trial time during cued navigation trials by FASD diagnostic group in version 2 of the CG Arena water maze task in the CT-L cohort. Values are adjusted for the covariates age at testing, prenatal cigarette exposure and mother's age at delivery. No significant differences were detected between diagnostic groups.

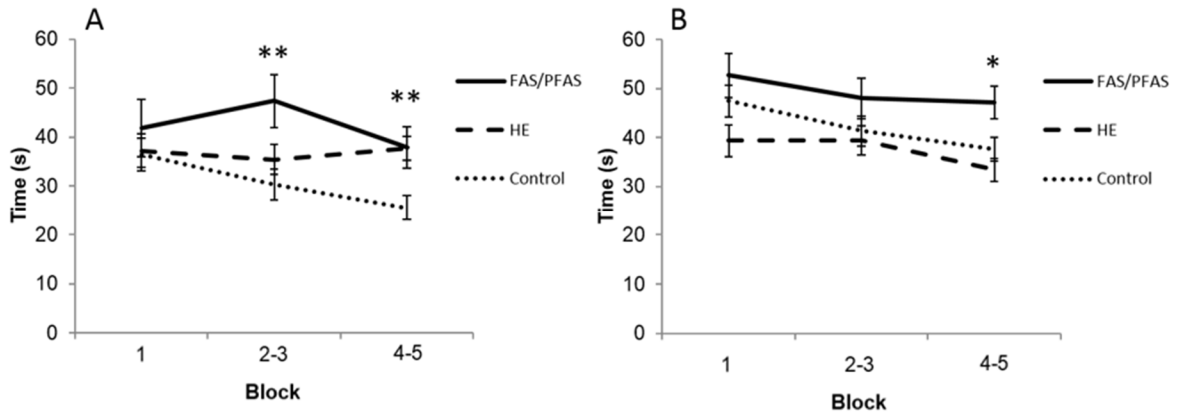

Figure 15. Mean (± S.E.) trial time to locate the hidden platform during acquisition by FASD diagnostic group for boys (A) and girls (B) in the CT-CS cohort. Values are adjusted for covariates in the model; age at testing and maternal age at delivery. Post-hoc analysis revealed that boys (A) with FAS/PFAS took significantly longer to find the hidden platform than the HE group and controls in blocks 2-3 and both FAS/PFAS and HE took longer than controls on blocks 4-5. For girls (B), the FAS/PFAS group took longer to find the hidden platform than the HE group on blocks 4-5. *$p < .05$ **$p < .01$.

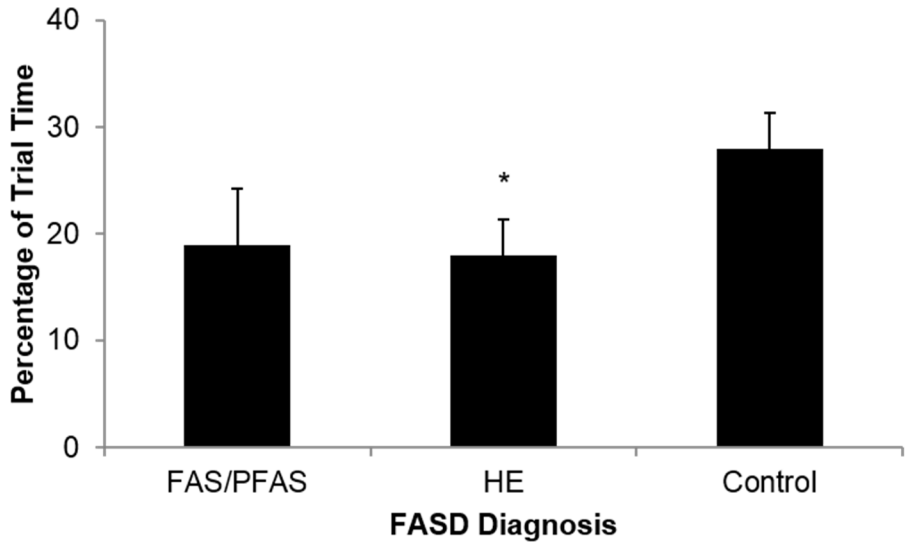

Figure 16. Mean (+ S.E.) percentage of time spent in the platform quadrant during the probe trial by FASD diagnostic group in the CT-CS cohort. Values are adjusted for the covariate age at testing. Children in the HE group spent significantly less time searching the platform quadrant than controls. *$p < .05$.

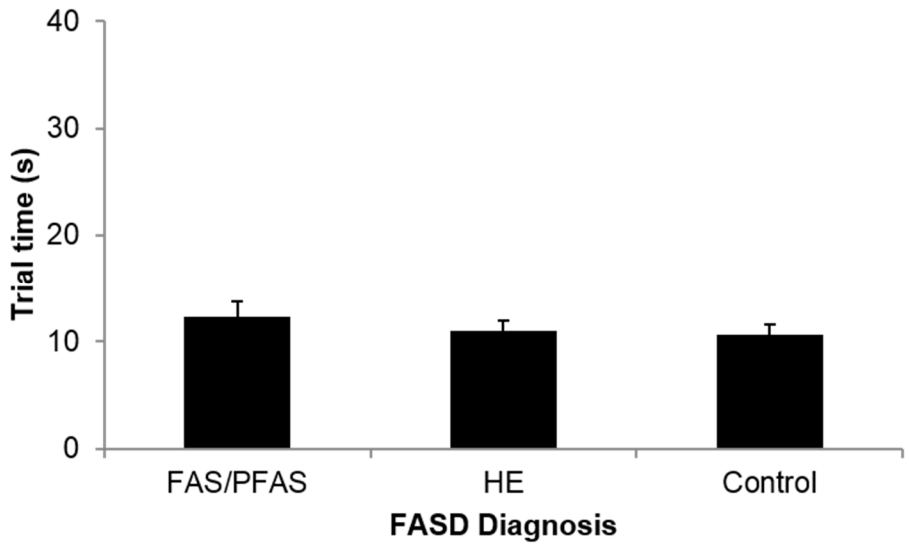

Figure 17. Mean (+ S.E.) trial time during cued navigation trials by FASD diagnostic group in the CT-CS cohort. Values are adjusted for the covariates age at testing, primary caregiver's marital status, and prenatal cigarette exposure. No significant differences were detected between diagnostic groups.

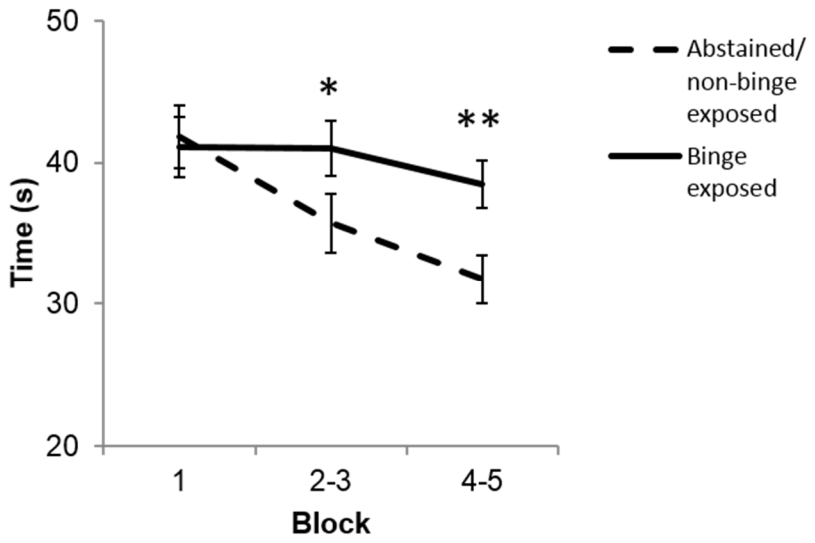

Figure 18. Mean (± S.E.) trial time to locate the hidden platform during acquisition comparing those non-exposed and non-binge exposed to those binge exposed in the CT-CS cohort. Values are adjusted for covariates in the model: mother's age at delivery and age at testing. Follow-up t-tests revealed significant differences between the groups in blocks 2-3 and 4-5. *$p < .05$ **$p < .01$.

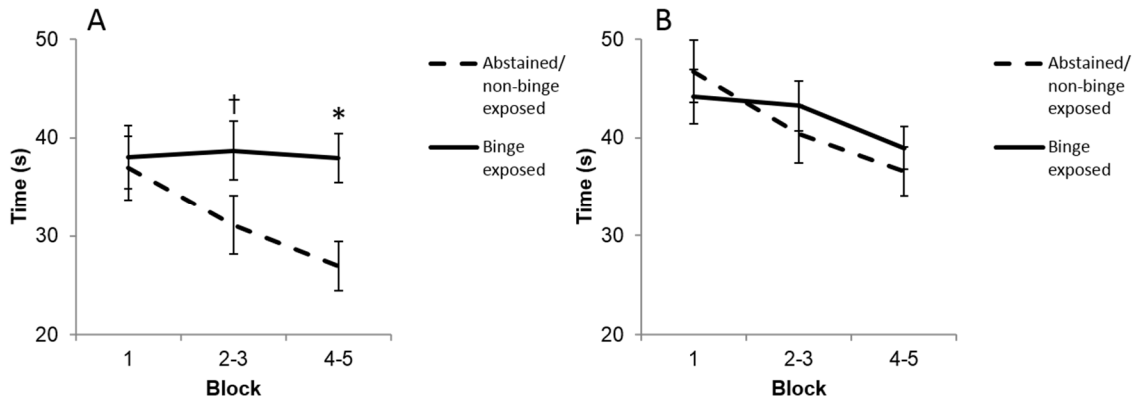

Figure 19. Mean (± S.E.) trial time to locate the hidden platform during acquisition comparing those non-exposed and non-binge exposed to those binge exposed in the CT-CS for boys (A) and girls (B). Values are adjusted for covariates in the model; age at testing, and maternal age at delivery. Follow-up t-tests revealed significant differences between the groups in blocks 2-3 and 4-5 for the boys (A). For girls (B), no differences between groups were detected. $^{\dagger}p < .10$ $^{*}p < .01$.

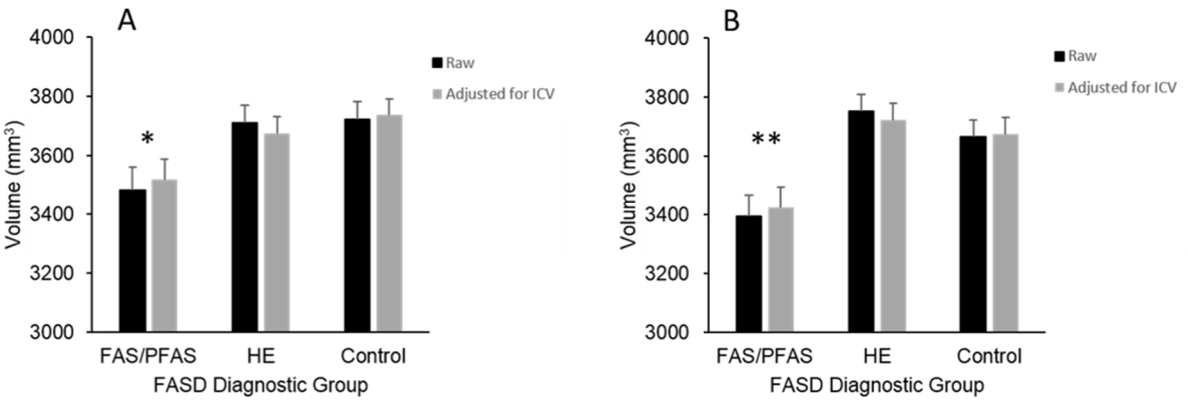

Figure 20. Mean left (A) and right (B) hippocampal volume by FASD diagnostic group for both raw hippocampal volumes and volumes adjusted for intracranial volume in the CT-L cohort. The FAS/PFAS group had significantly lower hippocampal volume than the HE and control groups. *$p < .05$ **$p < .01$.

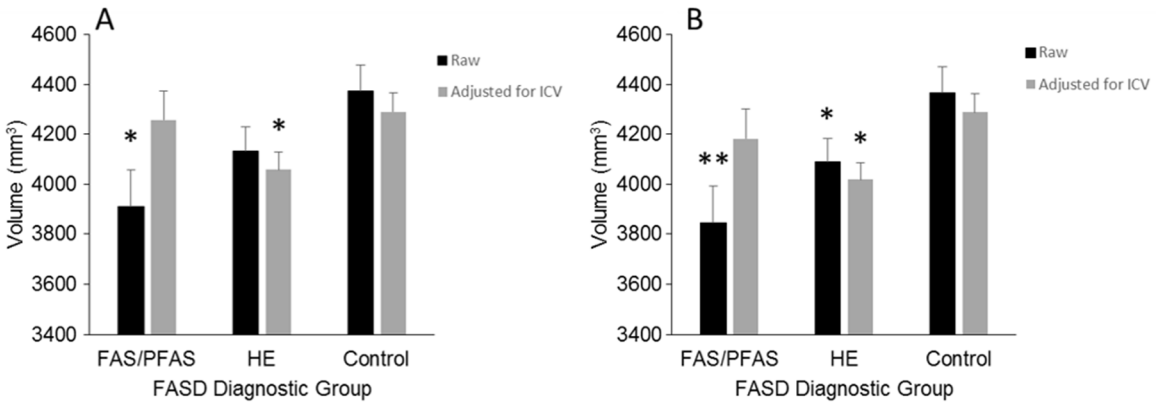

Figure 21. Mean left (A) and right (B) hippocampal volume by FASD diagnostic group for both raw hippocampal volumes and volumes adjusted for intracranial volume in the CT-CS cohort. The FAS/PFAS group had significantly smaller left and right hippocampal volumes than the control group. The HE group had smaller right hippocampal volume than the control group. After adjusting for intracranial volume, only the HE group differed significantly from controls. *$p < .05$ **$p < .01$.

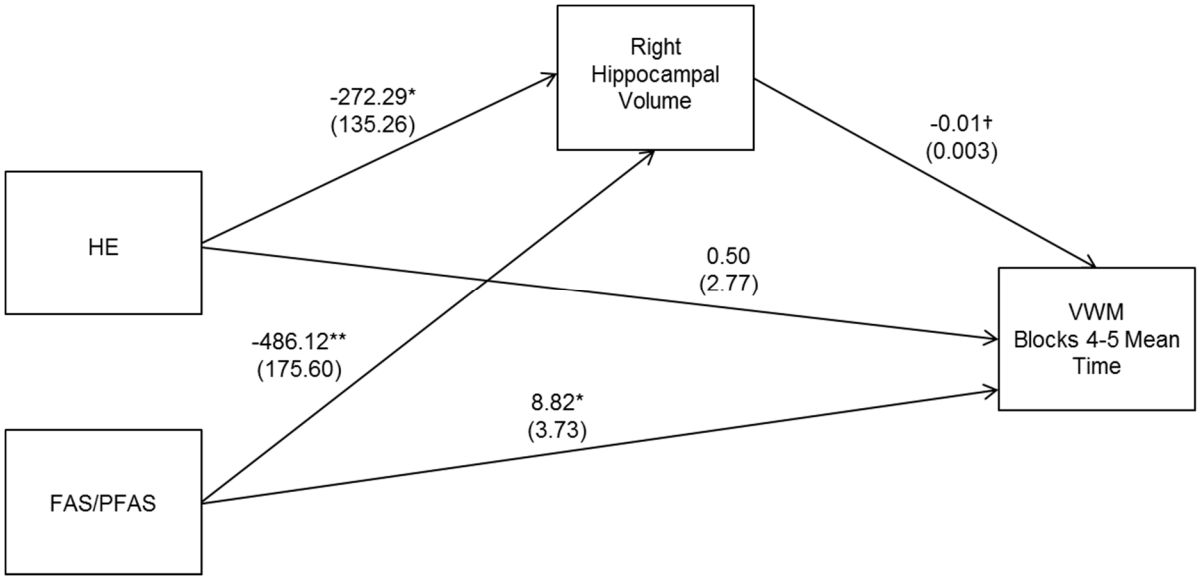

Figure 22. Path model showing the mediation of right hippocampal volume on the relation between FASD diagnostic group and mean trial time during blocks 4 and 5 of the VWM in the CT-CS cohort. Values are unstandardized regression coefficients (S.E.) adjusted for sex. The control group was used as the reference group for HE and FAS/PFAS coefficients. Both of the indirect effects of FAS/PFAS (effect = 2.66, 95% CI = 0.34, 6.69) and HE (effect = 1.49, 95% CI = 0.04, 4.85) on VWM performance through right hippocampal volume were significant. $^{\dagger}p < .10$ $^{*}p < .05$ $^{**}p < .01$.

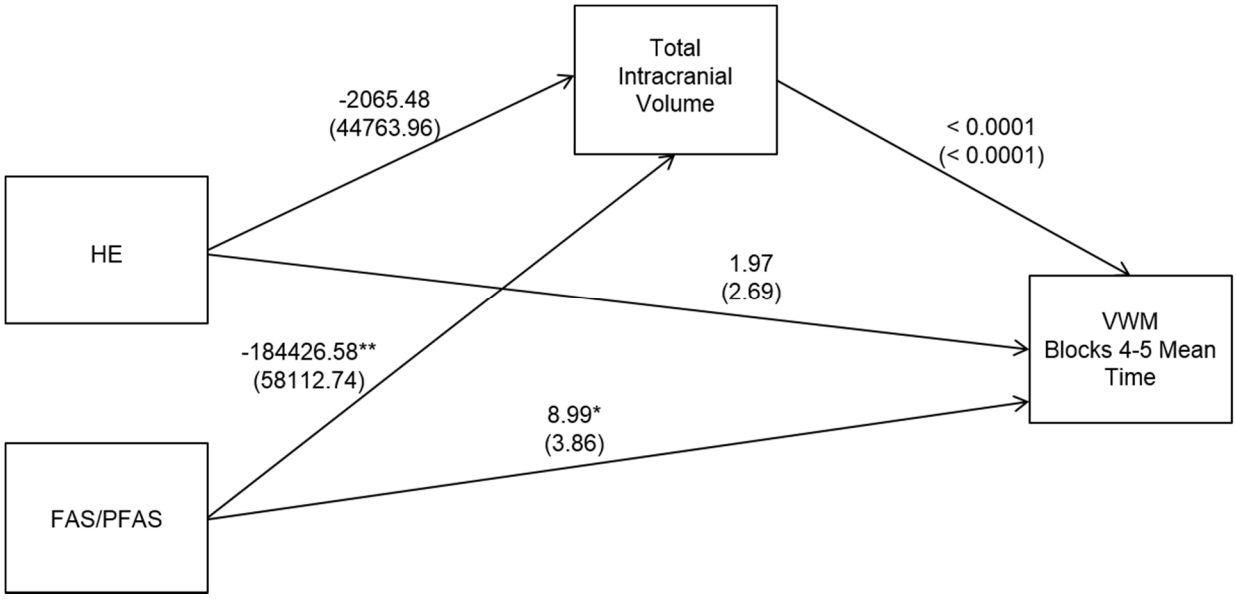

Figure 23. Path model testing the mediation of total intracranial volume (TIV) on the relation between FASD diagnostic group and mean trial time during blocks 4 and 5 of the VWM in the CT-CS cohort. Values are unstandardized regression coefficients (S.E.) adjusted for sex. The control group was used as the reference group for HE and FAS/PFAS coefficients. Neither the direct effect of TIV on VWM performance nor the indirect effects of FAS/PFAS (effect = 2.49, 95% CI = -0.64, 8.43) and HE (effect = 0.03, 95% CI = -1.25, 1.67) on VWM performance through TIV were significant. *$p < .05$ **$p < .01$.

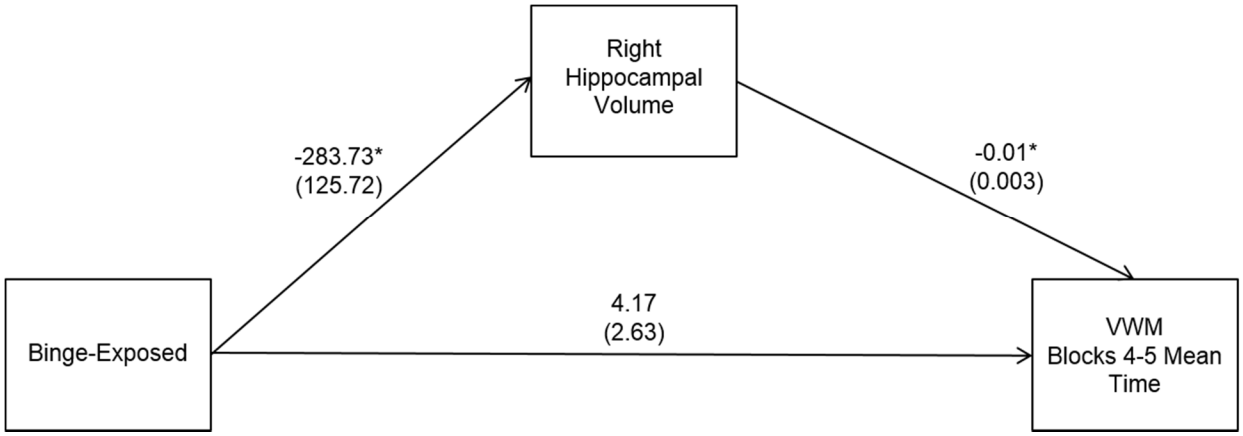

Figure 24. Path model showing the mediation of right hippocampal volume on the relation between prenatal alcohol binge exposure and mean trial time during blocks 4 and 5 of the VWM in the CT-CS cohort. Values are unstandardized regression coefficients (S.E.) adjusted for sex. The indirect effect of binge exposure on VWM performance through right hippocampal volume was significant (effect = 1.78, 95% CI = 0.21, 4.73). *p < .05.

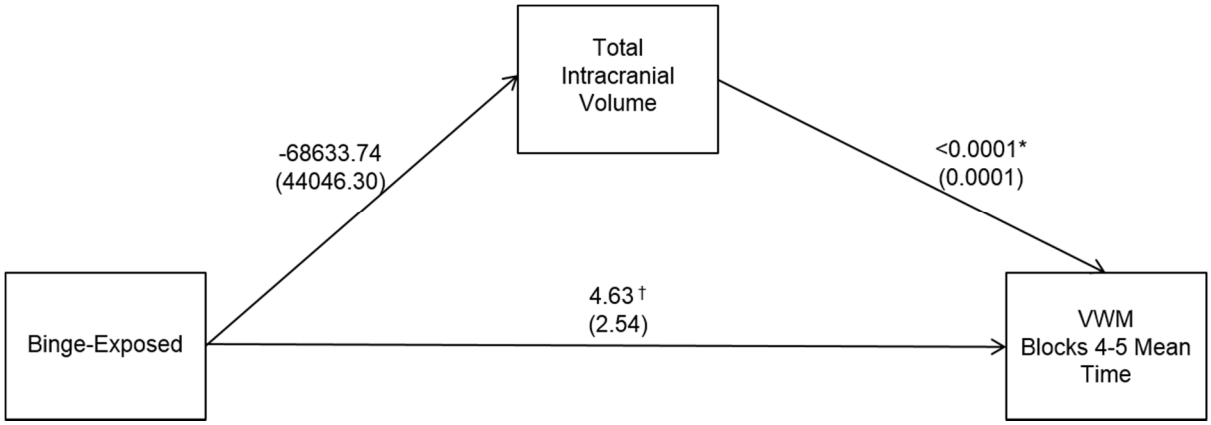

Figure 25. Path model testing the mediation of total intracranial volume (TIV) on the relation between prenatal alcohol binge exposure and mean trial time during blocks 4 and 5 of the VWM in the CT-CS cohort. Values are unstandardized regression coefficients (S.E.) adjusted for sex. The indirect effect of prenatal alcohol binge exposure on VWM performance through TIV was not significant (effect = 1.32, 95% CI = -0.05, 4.62). $^{\dagger}p < .10$ $^{*}p < .05$.

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ABSTRACT

AN ANALYSIS OF VIRTUAL PLACE LEARNING/NAVIGATION IN CHILDREN AND YOUNG ADULTS PRENATALLY EXPOSED TO ALCOHOL

by

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Fetal alcohol spectrum disorder refers to the spectrum of disorders resulting from prenatal alcohol exposure and is the leading cause of preventable mental retardation. Rodent studies have found that prenatal alcohol exposure impairs performance on the Morris water maze. This task requires the rodent to use distal room cues to locate a hidden platform in a pool of opaque water. Successful performance on this task is dependent upon hippocampal function. Rodents prenatally exposed to alcohol are impaired on the Morris water maze and show damage to hippocampal neurons. A human analogue of the Morris water maze, the virtual water maze has been created using computer-generated 3D virtual environments. Only one study has been conducted examining performance on the virtual water maze and FASD. This dissertation examined performance on the virtual water in three cohorts of individuals prenatally exposed to alcohol from Detroit and Cape Town, South Africa. Hypotheses were that children with a diagnosis of fetal alcohol syndrome or partial fetal alcohol syndrome, and those with a known history of prenatal alcohol exposure, but lacking the characteristic facial features will be impaired on the virtual water maze. Second, the amount of prenatal alcohol exposure will be negatively correlated with virtual water maze performance. Third, fetal alcohol-related reductions in hippocampal volume will mediate the relationship between FASD and virtual water maze performance. Lastly, prenatal alcohol related changes in testosterone will also mediate the relation between FASD and virtual

water maze performance. Results indicated that both those with an FASD diagnosis were impaired on the virtual water maze. Degree of prenatal alcohol exposure was also correlated with poorer performance on the virtual water maze. These results were detected in the cohort with the heaviest levels of prenatal alcohol exposure. Right hippocampal volume was shown to be a mediator of the relation between FASD/prenatal alcohol exposure and virtual water maze performance. Testosterone was not related to virtual water maze performance. These data demonstrate that virtual water maze performance is sensitive to the effects of heavy prenatal alcohol exposure. Furthermore, impairments on this task may be due to fetal alcohol-related damage in the hippocampus.

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