

## Spatial Navigation Abilities of Children Prenatally Exposed to Alcohol

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### **Abstract**

Numerous previous studies suggest that children with fetal alcohol spectrum disorders (FASD) perform poorly on a range of cognitive and behavioral tasks, including tests assessing spatial navigation. Previous studies of spatial navigation deficits have, however, been limited to male participants with FAS diagnoses only. The current study's sample included both males and females, with different levels of alcohol exposure, ranging from heavily exposed to non-exposed. All children were administered a desktop-based virtual navigation task that previous studies have shown is sensitive to deficits in cognitive map-based wayfinding and to hippocampal dysfunction. The results indicated that cued navigation was not affected by prenatal alcohol exposure, but that, on map-based navigation tasks (a) exposed boys performed more poorly than non-exposed boys, (b) girls generally performed more poorly than boys, and (c) there were no statistically significant differences between female exposed and non-exposed participants. These results are consistent with previous findings in the field, but add to the literature by suggesting that males may be more sensitive than females to hippocampal damage following prenatal alcohol exposure.

**Key words:** fetal alcohol syndrome; fetal alcohol spectrum disorders; teratogenic effects; hippocampus; spatial navigation; CG Arena.

## Introduction

The effects of prenatal alcohol exposure on the developing fetus are well established and exist along a continuum of physical abnormalities and behavioural and neurocognitive deficits (Spadoni, McGee, Fryer, & Riley, 2007). These deficits are collectively termed *fetal alcohol syndrome (FAS)* by Jones and Smith (1973) to denote a triad of characteristics that today are used by dysmorphologists as the basis of FAS diagnoses. The three defining features of FAS are growth deficiency, often manifested as below average height and microcephaly, evidence of central nervous system disorders, and a distinctive set of craniofacial anomalies (Mattson, Schoenfeld, & Riley, 2001). The term *fetal alcohol spectrum disorders (FASD)* has been coined recently as a non-diagnostic umbrella term for the range of deficits resulting from prenatal alcohol exposure (Spadoni et al., 2007). The term FASD therefore refers collectively to diagnoses of children who lack the characteristic facial anomalies of FAS, but who have either been labelled as having *partial fetal alcohol syndrome (PFAS)*, *alcohol-related neurodevelopmental disorder (ARND)*, or *heavy exposure to alcohol (HE)*; Jacobson et al. (2008); Mattson et al., 2001).<sup>1</sup>

In the Western world, FAS is the leading identifiable cause of mental retardation amongst children, and prenatal alcohol exposure is recognized as a causal factor in a range of developmental disorders (Burd, Klug, Martsolf, & Kerbeshian, 2003). Because a large proportion of children prenatally exposed to alcohol do not meet the criteria for FAS, it is vital that a behavioural phenotype for FASD be developed in order to assist in diagnosing children who lack the distinctive facial characteristics of FAS. Accurate diagnosis is vital in order for appropriate interventions to be put into place so that children with FASD might be able to live more functional lives.

### Functional Impairments in FAS and FASD

Children with FAS have been reported to have impaired general intellectual functioning, with average Full Scale IQs estimated to be in the low 70s (Mattson et al., 2001).

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<sup>1</sup> PFAS is characterised by the presence of two alcohol-related facial anomalies and one of the following deficits: small head circumference, growth retardation, or some degree of cognitive and/or behavioural dysfunction. ARND is characterised by significant cognitive impairment but a lack of facial anomalies. HE is characterised by a history of heavy prenatal alcohol exposure without the presence of alcohol-related facial anomalies or growth retardation (Jacobson et al., 2008).

Children diagnosed along the FASD continuum have more mildly impaired general intellectual functioning, with IQ scores not affected as severely (Mattson et al., 2001).

Numerous studies suggests that particular deficits in specific cognitive and motor domains might result from prenatal alcohol exposure and, importantly, may be independent of IQ impairments (Burd et al., 2003; Dodge et al., 2009; Mattson et al., 2001; Uecker & Nadel, 1996). For instance, children with FAS and FASD are impaired on an array of tasks that require higher-level executive functioning abilities (Mattson et al., 2001). Kodituwakku (2007) argues that prenatal alcohol exposure disrupts performance on cognition-based tests of executive functioning (e.g., the Tower of London), as well as on emotion-based tests of executive functioning (e.g., the Iowa gambling task). A disruption in neural circuitry is thought to be responsible for the deficits in goal-oriented behaviour (e.g., impaired planning disinhibition of responses, poor mental flexibility, and problem-solving difficulties) evident in children diagnosed with FAS.

Empirical research further suggests that although both verbal and non-verbal learning and memory are affected by alcohol's teratogenic effects, deficits in the processing of non-verbal material are more pronounced (Mattson et al., 2001). In a study of object and spatial memory, Uecker and Nadel (1996) tested 30 Native American school-aged children, 15 of whom had been diagnosed with FAS. The results indicated that the participants with FAS recalled fewer items on a delayed object location recall test, performed more poorly on maze navigation, and had more difficulty reproducing drawings than their matched healthy controls. The authors suggested that poor performance on the object location task and maze navigation, in particular, were indicative of poor spatial memory associated with prenatal alcohol exposure.

In a prospective study of 500 children prenatally exposed to alcohol, Streissguth, Barr, Sampson, and Bookstein (1994) found deficits in spatial memory across the continuum of FAS and FASD, although the Stepping Stone Maze (SSM) was found to be the most sensitive indicator of prenatal exposure to alcohol (PEA). Similarly, impaired visual-spatial memory was found in a sample of adolescents diagnosed with PEA who were compared to healthy controls and to a special education group (Platzman, Friedes, Lynch, & Falek, 2000, as cited in Manji, Pei, Loomes, & Rasmussen, 2009). Further congruent evidence for spatial memory deficits related to prenatal alcohol exposure are detailed by Manji et al. (2009) in their review of memory impairments in children with FASD.

In contrast to findings from the above-mentioned studies, no association between prenatal alcohol exposure and visual-spatial learning was found in a longitudinal study

investigating a cohort of 580 children prenatally exposed to alcohol (Willford, Richardson, Leech, & Day, 2004). The results of this latter study suggested that moderate exposure is related to generalised learning and memory deficits, as well as to specific auditory and verbal memory deficits, but not to visual-spatial memory deficits. The contrasting results across different studies suggest that the teratogenic effects of alcohol on the developing brain are not clear-cut with regard to spatial cognition, and that this topic needs to be studied further.

One important aspect of spatial cognition, and one that builds on basic spatial learning and memory abilities, is spatial navigation. The human ability to explore and navigate through space is believed to lie, at least partially, in a neurally-based cognitive mapping system. This system allows individuals to acquire representations of the relationships between the stimuli situated around them and, using these relations, create a cognitive map of their environment (O'Keefe & Nadel, 1978). Cognitive mapping abilities have received recent attention due to growing research into the complex neural networks needed for spatial navigation.

As a result of this growing interest, it has been shown that this system is particularly vulnerable to prenatal alcohol exposure. Although numerous studies have been conducted investigating spatial navigation abilities in healthy children, very little research on this topic exists for children prenatally exposed to alcohol. The majority of our current knowledge on the effects of alcohol on spatial navigation is based on animal studies, many of which have employed the Morris Water Maze (MWM; Morris, 1984), a task originally developed to test rodent abilities to spatially map the surrounding environment. The task typically requires rodents to swim in a tank of opaque water and to use distal cues (i.e., pictures on walls around the tank) to navigate toward a submerged platform that will free them from the water (Morris, 1984). Results from multiple trials in the MWM suggest that rodents prenatally exposed to alcohol perform significantly more poorly on the task than matched controls (Berman & Hannigan, 2000). It has been noted, however, that in both alcohol-exposed rats and healthy controls, significant differences in performance exist between the sexes, with females generally performing more poorly than males (D'Hooge & De Deyn, 2001).

In an attempt to replicate these results in humans, Hamilton, Kodituwakku, Sutherland, and Savage (2003) investigated the spatial navigation abilities of children diagnosed with FAS. Eight adolescent males diagnosed with FAS were compared to age- and sex-matched controls using a computerised version of the MWM known as the Virtual Morris Water Task (VMWT; Astur, Ortiz, & Sutherland, 1998). The researchers thought it appropriate to apply this task to FAS children as a previous study indicated a near-perfect

correlation between the responses of rats and humans to comparable manipulations of distal cues (Hamilton, Driscoll, & Sutherland, 2002). When performing the task, all participants were placed in a virtual swimming pool which had four distal cues placed on the walls around it. The experiment consisted of three phases. In phase I, a platform was hidden in a single location in the north-east quadrant of the pool for 20 trials. On each trial, the starting location was randomly selected, and participants were given 60 seconds to move from that location to the hidden platform.

In phase II, the platform was removed from the pool for a single 45-second trial. This probe trial allowed the researchers to examine the participants' search strategies when not receiving feedback. In phase III, 8 trials were conducted with the platform visible. The purpose of this latter phase was to control for motivation and attention, as the researchers hypothesised that, unless motivation and attention varied across the groups, the FAS and control participants would perform equally well when the platform was visible.

Results suggested that, in phase I, the FAS participants consistently used longer paths to navigate to the hidden platform. In phase II, when the platform was removed, the FAS participants took significantly longer paths to enter the platform quadrant than the controls, spent less time in the platform quadrant, and made significantly greater heading errors than the control participants. As predicted, there were no significant between-group differences on the phase III cued navigation trials.

Non-verbal intelligence of all participants in the Hamilton et al. study was measured using Raven's matrices. Results of these measures suggested that, on average, FAS participants had significantly lower non-verbal intelligence than controls, but that, contrary to expectations, higher non-verbal IQ in the FAS group predicted longer path lengths in phase I. These results suggest that poor place learning in the VMWT may be observed independently of low overall cognitive ability, suggesting that poor place learning is a result of damage to specific brain regions rather than from diffuse, non-specific damage (Hamilton et al., 2003).

Although the Hamilton et al. (2003) study made good contributions to knowledge about the spatial navigation aspect of the FAS behavioural phenotype, little is known about spatial navigation abilities in less severe cases along the FASD continuum. It seems that a dose-response relationship may exist between maternal alcohol consumption and neurobehavioural outcome (Berman & Hannigan, 2000; Carter et al., 2005), although the exact impact of dose, timing and duration of exposure on brain development is unclear.

Hamilton et al. (2003) also limited their sample to males, and it therefore remains unclear what effect alcohol has on the sex difference in spatial navigation abilities. As with

rodents, it is important to note that impairments in place learning may be difficult to detect in human females exposed to alcohol, as healthy control females, both pre-pubertal and adolescent, display relatively poor place learning abilities when compared to age-matched males (Astur et al., 1998; Newhouse, Newhouse, & Astur, 2007; Sandstrom, Kaufman, & Huettel, 1998). We included females in the current study sample in order to determine whether prenatal alcohol exposure reduced or enhanced the sex difference in performance evident in previous studies.

### **Correlation between Maternal Drinking and Neurobehavioural Outcome**

To my knowledge, few prospective studies exist that link accurate reports of maternal alcohol consumption during pregnancy with repeated assessments of neurobehavioural outcomes in offspring of those mothers. In their study, Jacobson, Chiodo, Sokol, and Jacobson (2002) suggested that in-depth antenatal interviews provide highly accurate depictions of maternal alcohol consumption (concerning volume and timing), thereby allowing accurate predictions of neurobehavioural deficits to be made. Analysis of their results suggested that 7 drinks per week on average are sufficient to be associated with adverse effects on development. Later studies confirmed these results and further suggested that episodes of binge drinking (characterised by > 4 drinks per occasion) are associated with greater neurobehavioural dysfunction than regular, lower alcohol-volume drinking (Dodge et al., 2009; Streissguth et al., 1994).

First-trimester binge drinking, in particular, has been associated with significant deficits in verbal memory, although no association has been found between alcohol consumption and spatial learning (Willford et al., 2004). These contradictory findings may be due to the fact that, in the study cited above, measures of visuospatial learning were limited to face and dot location subtests of the Children's Memory Scale.

It is currently unclear whether a dose-response relationship exists between prenatal alcohol exposure and spatial cognition ability in the developing child/adolescent. One of the aims of the current study was to fill this knowledge gap.

### **Neural Correlates of Spatial Navigation Deficits in FASD Children**

Studies using both animal and human participants have documented the impact of alcohol on several developing brain regions. For instance, Archibald et al. (2001), in a structural magnetic resonance imaging (sMRI) study, compared 14 children diagnosed with FAS and 12 children diagnosed with PEA to a group of 41 healthy control participants. Apart

from a decrease in overall brain size, the most significant structural differences between the FAS participants and controls were reduced basal ganglia volume (particularly in the caudate nucleus) and reduced parietal lobe volume. Structural imaging of heavily alcohol exposed children has also revealed abnormal thinning of the corpus callosum and, in extreme cases, complete agenesis of the corpus callosum (Dodge et al., 2009; Mattson et al., 2001).

Studies of the neural bases of the cognitive mapping system have indicated that intact connections between several brain regions are necessary for optimal spatial navigation. Animal studies have, for example, implicated damage to cerebellar, basal forebrain, striatal, and hippocampal regions in poor MWM performance in rats. The same regions have been documented in several human and animal studies as being extremely sensitive to prenatal alcohol exposure (Archibald et al., 2001; D’Hooge & De Deyn, 2001; Spadoni et al., 2007). Mattson et al. (2001), for example, connected prenatal alcohol exposure to cerebellar damage, and in particular, damage to the anterior portion of the vermis. In general, cerebellar damage is proposed to contribute to the deficits in fine motor functioning, poor attention, and impaired conditioning evident in children diagnosed with FAS (Spadoni et al., 2007). Petrosini, Molinari, and Dell’Anna (1996) suggested that the role of the cerebellum in spatial learning is therefore primarily procedural; in other words, cerebellar processing is responsible for the procedures needed to locate a target, rather than the memory for its spatial location. It has also, however, been suggested that the cerebellum plays a role in motivating an individual to explore a novel environment (Caston et al., 1998).

In their review of MWM literature, D’Hooge and De Deyn (2001) provided evidence from animal studies, which have identified basal forebrain damage as being responsible for the most severe hidden platform and probe trial performance deficits seen in rats. They suggest that this responsibility can be placed on the basal forebrain because it is one of the major sources of innervation for the hippocampus, a region critical for spatial navigation.

A wealth of evidence exists portraying the high vulnerability of the hippocampus, a sub-cortical region essential for human memory and learning, and, in particular, spatial navigation (Maguire, Frackowiak, & Frith, 1996; O’Keefe & Nadel, 1978), to alcohol exposure (Astur, Taylor, Mamelak, Philpott & Sutherland, 2002; Berman & Hannigan, 2000). Because hippocampal cells are believed to be the primary substrate underlying place learning (Blum & Abbott, 1996; Nadel, 1991; O’Keefe & Dostrovsky, 1971), it has been suggested that poor performance during hidden platform trials in the VMWT is a result of damaged hippocampal cells (D’Hooge & De Deyn, 2001). In their review of the teratogenic effects of alcohol, Guerri, Bazinet, and Riley (2009) argue that during weeks 7 to 20 of gestation,



alcohol exposure reduces the number of neurons and glial cells in the hippocampus. Exposure at this time may therefore account for deficits in spatial navigation in FAS children.

In summary, the growing body literature on FASD has contributed significantly to the development of a cognitive/behavioural phenotype for children prenatally exposed to alcohol. The spatial navigation aspect of the phenotype remains relatively unexplored, however. Although previous studies of spatial navigation and prenatal alcohol exposure have provided valuable insights, the results are limited due to (a) the use of children diagnosed with FAS only, (b) small sample sizes, and (c) samples limited to males only. The current study is therefore valuable in that it attempted to overcome the limitations of previous studies as well as contribute to the behavioural phenotype of FASD.

The current study therefore tested the following hypotheses:

(1) Following Hamilton et al. (2003), children prenatally exposed to alcohol will not perform significantly more poorly than non-exposed children on cued navigation trials of a spatial navigation task.

(2) Children prenatally exposed to alcohol will perform significantly more poorly than non-exposed children on a spatial navigation task that assesses cognitive mapping ability.

(3) Performance on the cognitive mapping task will differ significantly within the exposed sample (i.e., across FAS, partial FAS, and HE participants)

(4) Girls, both exposed and non-exposed, will perform significantly worse than boys on that task.

## **Methods**

### **Design**

The current study took the form of a quasi-experiment and had a cross-sectional design. I compared the performance of alcohol-exposed and non-exposed participants on spatial navigation tasks at a single point in time. I then did further analyses by separating the exposed children by diagnostic group (FAS, PFAS, and HE). The research assistant who administered the navigation tasks was blind to the diagnosis of each participant during administration.

### **Participants**

The sample consisted of 46 children born to Coloured women living in the Western Cape. All of the children are part of an ongoing prospective longitudinal study of the effects of prenatal alcohol exposure on development. The principal investigators obtained ethical

approval for the study from the Wayne State University Human Investigation Committee (reference number 099504B3F; see Appendix A). The mothers of the participants were recruited during pregnancy, between July 1999 and January 2002. Informed consent was obtained from each mother when recruited for the study. The principal investigators informed the mothers of the risks of drinking during pregnancy and advised them to stop. All of the mothers were also offered an intervention aimed at reducing drinking during pregnancy.

Shortly after recruitment, researchers conducted interviews with the mothers, using a timeline follow-back approach (Jacobson et al., 2002), to determine the incidence of drinking and amount of alcohol consumed around the time of conception and during pregnancy. The volume of alcoholic beverages consumed was recorded and converted into ounces of absolute alcohol (AA). Heavy drinking was defined as averaging at least 1.0 oz of AA (the equivalent of two standard drinks) per day or reporting having had at least two episodes of binge drinking (at least four drinks per occasion) during the first trimester of pregnancy. Any child whose mother met the abovementioned criteria was considered heavily exposed. All of the children born to heavy-drinking mothers were examined by FAS dysmorphologists and were given a diagnosis of FAS, PFAS, or HE.

Of the total sample used, 29 children were considered alcohol exposed. Five (11%) of these met the criteria for FAS, 13 (28%) met the criteria for PFAS, and 11 (24%) met the criteria for HE. The remaining 17 (37%) children were recruited as controls on the basis of their mothers averaging less than 0.5 oz AA/day and having reported no binge drinking episodes in the first trimester. The sample consisted of approximately 60% boys and 40% girls. This ratio did not differ significantly between the exposed and non-exposed groups,  $\chi^2(1, N = 46) = 1.51, p = .220$ . At the time of testing, the children ranged in age from 8 to 10 years,  $M = 9.30, SD = 0.39$ . The average age at testing did not differ significantly between the groups however,  $t(44) = 1.44, p = .156$ .

The intelligence of each child was measured at a testing session nine years after initial recruitment using the Wechsler Intelligence Scale for Children (WISC-IV; Wechsler, 2004). Scores had to be estimated for three children who were unable to complete all of the subtests. The mean IQ of the alcohol exposed children ( $67.77, SD = 9.33$ ) was statistically significantly lower than that of the non-exposed children ( $78.76, SD = 10.75$ ),  $t(44) = -3.65, p = .001$ . Figure 1 shows the distribution of IQ scores by group.

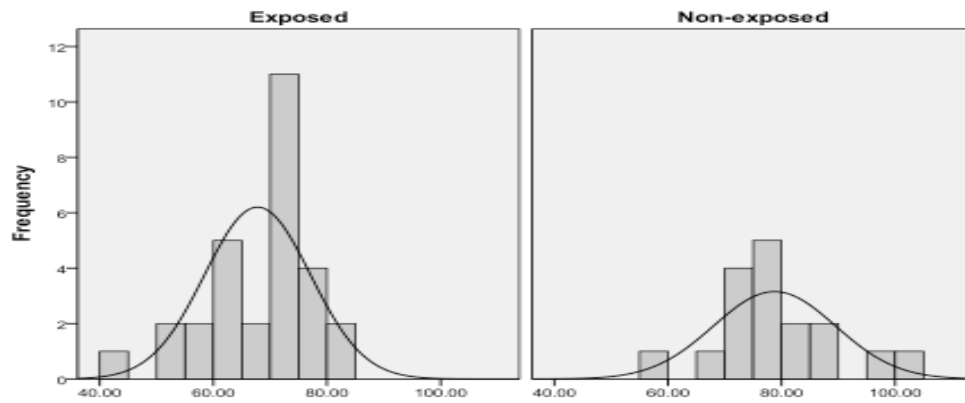


Figure 1. WISC-IV Full-Scale IQ distribution by group

The demographic and background characteristics of the mothers of the cohort children are summarized in Table 1. As can be seen, there were no statistically significant between-group differences with regard to age at time of delivery. Overall, the mothers in the cohort were relatively poorly educated, with an average of 8.8 years of education; only 8 (18.2%) had completed high school. Furthermore, there was a statistically significant between-group difference in terms of years of education, with drinking mothers having lower levels of education than non-drinking mothers. Figure 2 shows the distribution of maternal years of education by group.

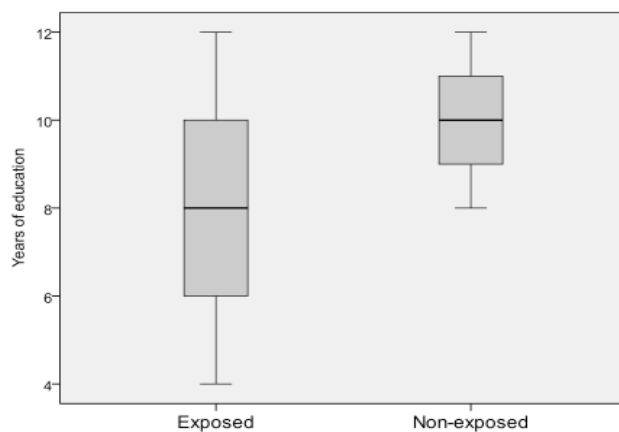


Figure 2. Distribution of maternal education (in years).

On average, the mothers scored poorly on the Standard Progressive Raven's Matrices test of non-verbal intelligence (Raven, 1966). There were also statistically significant between-group differences on this measure, with drinking mothers performing more poorly than non-drinking mothers.

The percentage of married mothers in the drinking group was statistically significantly lower than that in the non-drinking group. The Hollingshead scale of social class (Hollingshead, 1975) was used to describe the socioeconomic status (SES) of the mothers. Independent sample *t*-tests indicated that the drinking mothers had a significantly lower SES than the non-drinking mothers, despite both groups of participants being recruited for the study at the same clinic.

Finally, most of the mothers (69.57%) smoked during pregnancy, with 15.6% smoking an average of 10 or more cigarettes per day. There were, however, no statistically significant between-group differences in terms of the number of cigarettes smoked per day during pregnancy. Few mothers (4.3%) used marijuana, and none reported using cocaine or Mandrax during pregnancy.

Table 1.  
*Maternal Clinical and Sociodemographic Characteristics*

Measure	Exposed ( <i>n</i> = 29)	Non-exposed ( <i>n</i> = 17)	Test statistic	<i>p</i>	ESE
Age at delivery	26.52 (6.63)	25.61 (3.11)	0.63	.534	0.16
Education (years)	8.00 (2.65)	10.24 (1.20)	-3.91	< .001***	0.99
Raven's matrices score <sup>a</sup>	26.33 (10.88)	34.21 (8.19)	-2.38	.022*	0.77
Married (%)	20.70	58.80	6.87	.009**	0.39
SES group <sup>b</sup>	4.41 (0.78)	3.82 (0.73)	2.54	.015*	0.76
Prenatal cigarettes/day <sup>c</sup>	7.69 (5.25)	9.92 (14.78)	-0.36	.730	0.28

*Note.* The test statistic was either *t* or  $\chi^2$ . ESE = effect size estimate, in this case Cohen's *d*  
<sup>a</sup>Data missing for 5 mothers. <sup>b</sup>Based on Hollingshead Four Factor Index of Social Status Scale (Hollingshead, 1975). <sup>c</sup>Consumers only.

\**p* < .05, \*\**p* < .01, \*\*\**p* < .001

## Materials and Apparatus

### *Computer-Generated Arena (CG Arena)*

Custom-designed software was loaded onto a laptop computer, allowing the CG Arena to be displayed on the laptop monitor. The monitor displayed a multi-colored view of a circular arena situated within a square room. The view was in first-person perspective, as if the participant was standing on the arena floor. Each wall of the arena room was assigned one or more pictures; these served as distal cues during navigation. Figure 3 illustrates the first-person perspective of the pool seen by each participant. The arena was divided into four

imaginary quadrants of equal size. A square target was placed on the floor of the CG Arena and was initially visible to the participants.



*Figure 3.* Screen shot of CG Arena during an invisible trial

Following 5 visible target trials assessing cued navigation ability, 16 invisible target trials assessing cognitive mapping ability commenced. During each invisible target trial, the participant was placed in the arena facing a distal wall. The participant then pressed the space bar and began to navigate using the arrow keys. When the participant moved over the area occupied by the target, the target became visible and a tone sounded. The participants were given 2 minutes to locate the target before the trial automatically terminated. The participant's starting position on each invisible target trial was randomly selected, but the target remained in the same location in the north-west quadrant across the 16 trials.

Immediately following the set of invisible target trials, each participant received a single probe trial. The probe trial appeared to be identical to the invisible target trials except, unbeknownst to the participants, the target was removed from the arena. Once the time limit was reached, the probe trial terminated, signaling the end of the CG Arena task.

#### *Post-experiment questionnaire*

This questionnaire (see Appendix B) was used to assess the participants' previous experience of playing video games. It also asked participants about the strategies they used to locate the hidden target. Furthermore, it required the participants to give a subjective rating of the task difficulty, and asked whether they believed that the target remained in the same position throughout the experiment.

### *Maternal alcohol consumption data*

Data collected during maternal interviews regarding alcohol consumption during pregnancy was obtained from the principal investigators of the larger study within which this one is nested. A summary of these data is presented in Table 2. Of the 46 mothers recruited for the study, more than half (56.50%) reported drinking around the time of conception, with slightly more (65.20%) drinking throughout pregnancy. Of the mothers who reported drinking at conception and during pregnancy, 83.33% reported reducing their alcohol consumption during pregnancy, with an average reduction of 0.55 oz AA (an equivalent of just over 1 standard drink per day) and 0.79 oz AA (an equivalent of almost 2 standard drinks) per drinking day.

As Table 2 shows, average alcohol consumption per day at the time of conception differed significantly between the groups, with the drinking mothers having consumed significantly more alcohol per day than the non-drinking mothers. Similarly, alcohol consumption per drinking day at the time of conception differed significantly between the groups, with the drinking mothers having consumed significantly more alcohol than the non-drinking mothers. The proportion of drinking days around the time of conception was also significantly higher for the drinking mothers than the non-drinking mothers.

Table 2 also shows that similar patterns of alcohol consumption were found throughout pregnancy. Once again, the drinking mothers consumed significantly more alcohol per day than the non-drinking mothers, consumed significantly more per drinking day, and drank significantly more frequently than the non-drinking mothers.

During the maternal interviews, signs of alcohol abuse or dependence were noted and analysed in relation to criteria set out by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; APA, 2000). Fourteen of the 29 drinking mothers (48%) were found to have either abused or been dependent on alcohol at some point in their lives. Although alcohol consumption amongst the control mothers was negligible during pregnancy, 3 of those 17 mothers (18%) reported drinking behaviour that matched the DSM-IV criterion for past alcohol abuse or dependence. As Table 2 shows, there were statistically significant between-group differences with regard to the distribution of alcohol abusing or dependent mothers across groups, with a moderate effect size associated with the difference.

Table 2.  
*Maternal Alcohol Consumption*

	Exposed ( <i>n</i> = 29)	Non-exposed ( <i>n</i> = 17)	<i>t</i> / $\chi^2$	<i>p</i>	<i>ESE</i>
Daily average alcohol consumption					
At conception	1.31 (2.13)	0.005 (0.02)	3.29	.003**	0.75
During pregnancy	0.88 (1.38)	0.008 (0.03)	3.40	.002**	0.78
Average alcohol consumption per drinking day <sup>a</sup>					
At conception	3.66 (2.97)	0.07 (0.28)	6.47	< .001***	1.49
During pregnancy	3.49 (2.06)	0.16 (0.45)	8.36	< .001***	1.96
Proportion of drinking days per week <sup>a</sup>					
At conception	0.28 (0.23)	0.004 (0.02)	6.37	< .001***	1.48
During pregnancy	0.21 (0.19)	0.006 (0.02)	5.79	< .001***	1.32
Alcohol abusing or dependent <sup>b</sup> (%)	48.3	17.6	4.32	0.038*	0.31

*Note.* ESE= effect size estimate, based on Cohen's *d*. <sup>a</sup>Measured in oz AA. <sup>b</sup>Based on DSM-IV-TR criteria.

\**p* < .05, \*\**p* < .01, \*\*\**p* < .001

## Procedure

On the day of testing, a driver transported the mothers and children to the Child Development Research Laboratory at the UCT Health Science Campus. Before testing, all of the mothers provided written consent for the 9-year follow up study; each child also provided oral assent (see Appendix C).

Following these consent procedures, each participant entered the testing room and was seated at a desk with a computer displaying the CG Arena. The research assistant gave standardized verbal instructions to each participant, outlining what they were going to see on the computer screen, how to navigate around the CG Arena, and what the goal of the task was. Because the participants were Afrikaans-speaking, the instructions were given in Afrikaans. Immediately following completion of the CG Arena task, the research assistant filled out the post-experiment questionnaire and assessed the child's performance on the task.

At the end of the testing session, the investigators provided the mothers with R150 as compensation for their time and gave each child an educational toy. The 9-year follow-up cohort study was funded by the National Institute of Health (NIH) and National Institute of Alcohol Abuse and Alcoholism (NIAAA) in the United States (reference number R01AA016781).

## Statistical Analysis

I used the Statistical Package for the Social Sciences version 18 (SPSS, 2009) to analyze the data. Descriptive statistics were obtained for the sample characteristics, for the independent variable (alcohol exposure), and for the outcome variables (performance on various aspects of the CG Arena task). I inspected the distribution of the data on all variables to ensure that assumptions for parametric statistical tests were upheld.

Before performing inferential data analyses, I correlated each potential control variable with CG Arena performance, with alpha set at 0.10. Two maternal characteristics correlated significantly with CG Arena performance: years of education, and Raven's matrices scores. The main method of inferential data analysis was repeated-measures ANOVA. Because previous studies have indicated directional results for the invisible trials, the significance for these trials was 1-tailed. Because I expected no difference in performance during the visible trials, significance was analyzed 2-tailed. Throughout data analysis, alpha was set at 0.05. During the visible and invisible target trials, CG Arena performance was assessed by path length from start position to target. For the probe trial, however, performance was assessed based on time spent in the NW quadrant, as I assumed that participants who had successfully mastered place learning would spend the majority of their time searching the NW quadrant.

Repeated-measures ANOVAs were first run by comparing the performance of the exposed and non-exposed children on the visible trials. Because previous studies indicated a sex difference in performance, the analyses were run separately for boys and girls. Identical analyses were then run for the invisible trials, which were split into 4 blocks of 4 trials each.

After running analyses for exposed versus non-exposed participants, I then reran the model with sex as the between-subjects factor in order to determine whether a main effect for sex exists, regardless of alcohol exposure. In order to replicate previous studies, I then split the participants into their diagnostic groups and reran the model in order to compare the performance between heavily exposed syndromal, non-syndromal, and non-exposed boys.

When the aforementioned control variables were added to the repeated-measures ANOVA models as covariates, the significant between-groups differences found no longer existed. I therefore decided that, because previous studies did not control for such variables, I would not add these variables to the model, as it is likely that alcohol consumption is too highly inter-correlated with these variables to be able to tease their effects apart.

For probe trial performance, univariate ANOVA analyses were run in order to determine whether differences in time spent in the NW quadrant existed between the exposed



and non-exposed participants. Again, the results were analyzed separately for boys and girls. I then reran the model with sex as the between-subjects factor to determine whether a main effect for sex existed for probe trial performance.

Finally, I correlated the post-experiment questionnaire results with CG Arena performance in order to determine whether gaming experience, in particular, could account for any of the variance seen in CG Arena performance.

## Results

### Phase I: Cued Navigation

The paths of all participants were recorded across the 4 visible trials, which constituted the cued navigation phase (see Appendix D for descriptive statistics). Following the convention in this literature, the starting position for each visible platform trial was pseudorandomly selected as the participant pressed the spacebar key, thereby initiating the trial (Thomas, Hsu, Laurance, Nadel, & Jacobs, 2001). As a result, the path length to target varied for each trial, therefore the deviation from an optimal path length to target was recorded as a measure of visible trial performance. Figure 4 illustrates the mean deviation from an optimal path length for male and female exposed and non-exposed participants. Upon visual inspection, the figure suggests that both groups of boys, and the non-exposed girls, performed equally and with similar efficiency across the 4 trials. The exposed girls seemed to initially perform very poorly and then showed some improvement across trials 2 to 4, although their deviation from an optimal path length remained higher than those of the other participants.

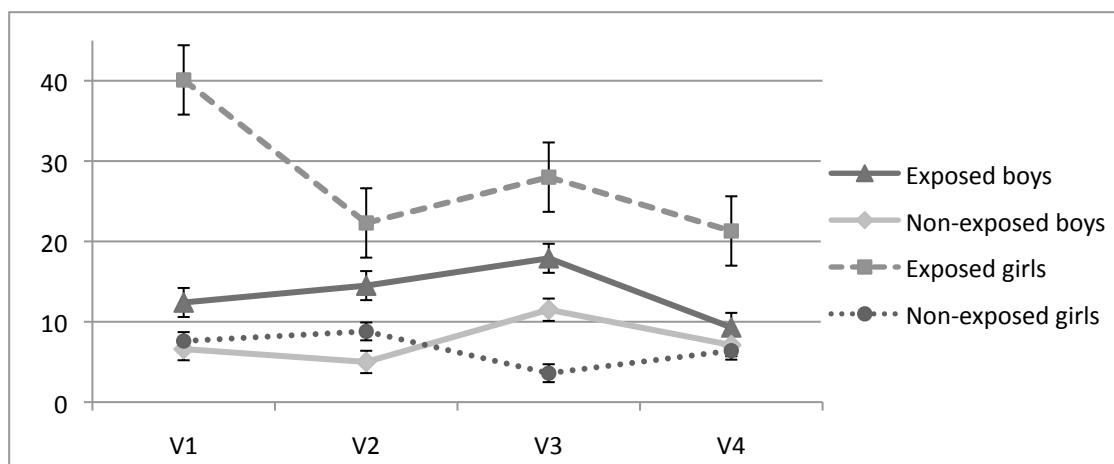


Figure 4. Mean deviation from an optimal path length on the set of four visible target trials.

Results from a repeated-measures ANOVA support this impression. With regards to deviation from an optimal path for boys, no significant within-subjects effect for trials was found,  $F(3, 75) = 1.12, p = 0.348$ , suggesting that performance did not improve across the trials. There was no statistically significant trial x group interaction,  $F(3, 75) = .052, p = .984$ , or main effect of exposure,  $F(1, 25) = 0.96, p = .338$ . These results suggest that the exposed and non-exposed boys performed similarly and with equal efficiency across the 4 visible target trials.

Similar results were found for girls: there was no statistically significant within-subjects effect of trials,  $F(3, 45) = 0.47, p = .707$ , no significant trial x group interaction,  $F(3, 45) = 0.45, p = .716$ , and no main effect of exposure,  $F(1, 15) = 2.45, p = .138$ . The effect size associated with the main effect for exposure amongst girls was relatively large, however, ( $\omega^2 = 0.14$ ), suggesting some degree of difference in performance between exposed and non-exposed girls.

Because some difference in performance was evident amongst the girls, a repeated-measures ANOVA was run to determine whether a main effect for sex existed. The results indicated no such statistically significant effect,  $F(1, 42) = 0.86, p = .359$ , suggesting that boys and girls performed equally efficiently on the set of visible target trials.

In summary, the results from this phase indicate that alcohol-exposed children are not significantly impaired on cued-navigation tasks, and that performance on such tasks is independent of sex.

## **Phase II: Place Learning**

The paths of all the participants were recorded across the 16 invisible platform trials. Following the convention in this literature (see, e.g., Astur et al., 1998; Hamilton et al., 2003; Jacobs et al., 1998), performance on each set of 4 invisible trials was averaged to create a trial block. The performance on the resulting 4 trial blocks were analysed in order to determine place learning abilities. In order to replicate the findings of Hamilton and his colleagues, repeated-measures ANOVAs were run separately for boys and girls.

Analyses of data from exposed and non-exposed boys indicated no statistically significant main effect of trial block,  $F(3, 75) = 1.16, p = .167$ , and no significant block x group interaction,  $F(3, 75) = 0.74, p = .266$ . There was, however, a significant main effect of group,  $F(1, 25) = 3.60, p = .035, \omega^2 = 0.13$ . These results suggest that exposed boys performed significantly more poorly than non-exposed boys across the blocks and that

performance did not change for either group across the blocks. Figure 5 illustrates the mean path length for each group of boys across the blocks.

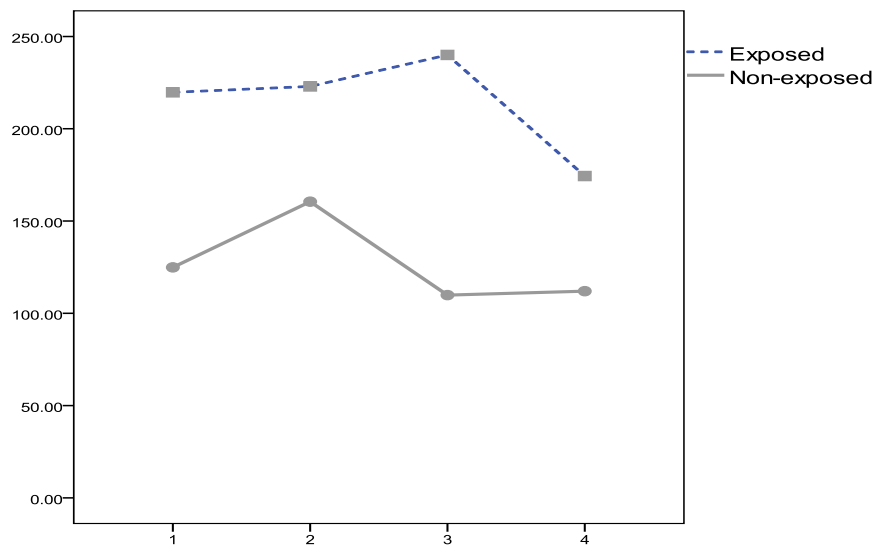


Figure 5. Mean path length for boys in the place learning phase

A similar repeated-measures ANOVA sought to determine whether there were sex differences in place learning performance. Figure 6 illustrates the data used in this analysis. There was no statistically significant main effect of trial block,  $F(3, 132) = 0.37, p = .389$ , and only a tendency toward statistical significance with regard to the main effect of group,  $F(1, 44) = 2.43, p = .063, \omega^2 = 0.05$ . Similarly, the trial block by group interaction approached significance,  $F(3, 132) = 2.06, p = .054, \omega^2 = 0.05$ . These results suggest that there was a strong tendency toward a sex difference in place learning performance across the blocks, with the difference becoming more evident in the later trials, where the boys noticeably improved their path lengths and the girls began to perform more poorly.

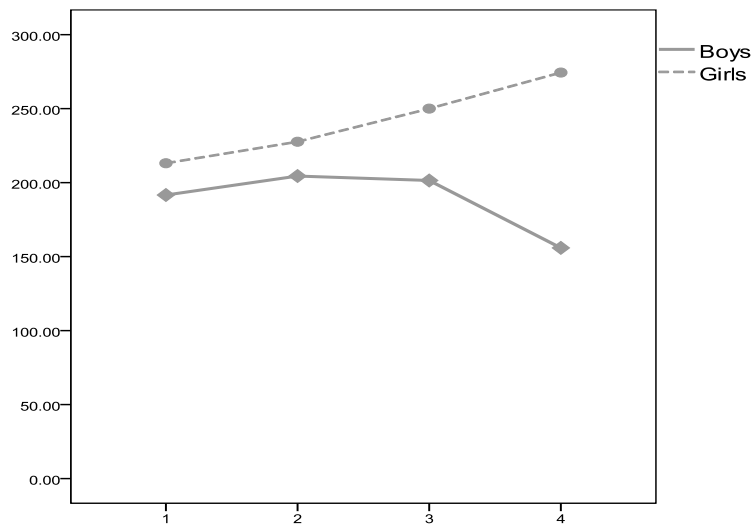


Figure 6. Mean path length for boys and girls across invisible trial blocks.

Because Figure 6 illustrated a difference in female place learning performance across the 4 blocks, I then ran a separate repeated-measures ANOVA on the data from exposed and non-exposed girls. This time, there was no statistically significant main effect of trial block,  $F(3, 51) = 0.83, p = .241$ , no significant trial block x group interaction,  $F(3, 51) = 1.01, p = .198$ , and no significant main effect of group,  $F(1, 17) = 0.45, p = .255$ . These data therefore suggest that exposed and non-exposed girls showed relatively equal performance, and that performance did not change significantly across the blocks for either group.

To extend the conclusions drawn by Hamilton et al. (2003), who studied FAS and control boys only, I ran a repeated-measures ANOVA using diagnostic group as a factor in order to determine whether significant differences in performance existed between boys with different levels of alcohol exposure. The heavily exposed FAS and PFAS boys were grouped together as “syndromal”, and were compared to the nonsyndromal HE boys and to non-exposed controls. Figure 7 presents the data used in this analysis.

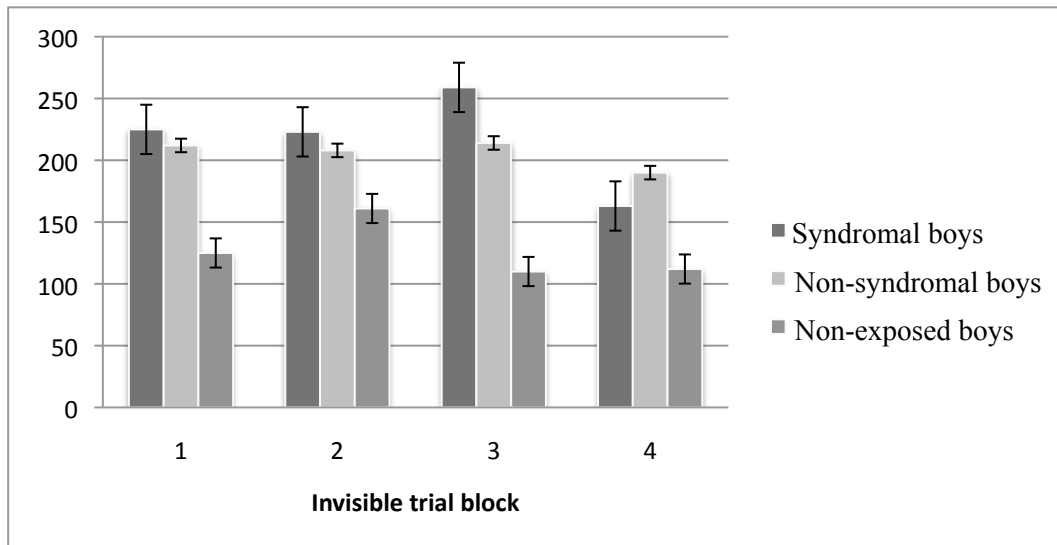
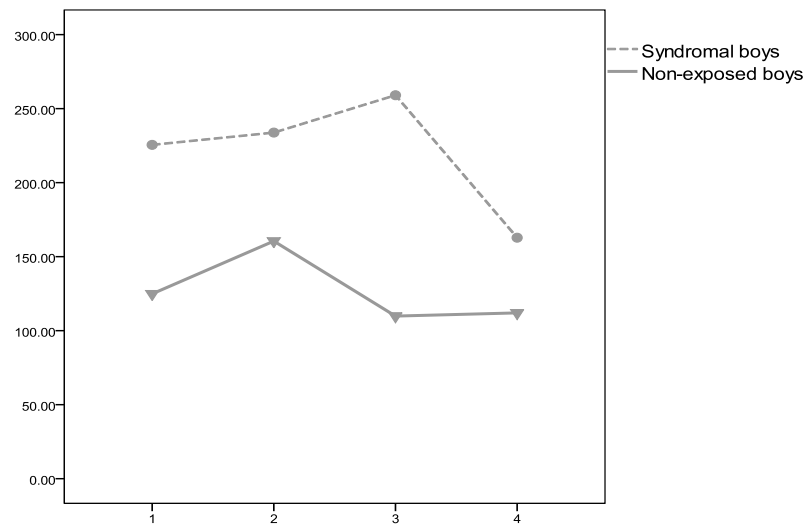


Figure 7. Mean path length for diagnostic groups of boys across invisible trial blocks.

The results indicated no statistically significant main effect of trial block,  $F(3, 72) = 1.35, p = .133$ , no significant trial block x group interaction,  $F(6, 72) = 0.64, p = .351$ , and no significant main effect of group,  $F(1, 24) = 1.77, p = .096$ . These results suggest that there were no differences in place learning performance between heavily exposed syndromal, nonsyndromal, and non-exposed boys. Visual inspection of Figure 7 suggests a noteworthy difference in path length between syndromal and non-exposed boys, however, with nonsyndromal boys demonstrating an intermediate level of performance. The absence of a significant between-subjects effect may therefore be the result of the intermediate path lengths obtained by the HE boys.

In order to determine whether a significant performance difference existed between heavily exposed syndromal and non-exposed boys, the model was then rerun with data from the HE boys removed. Figure 8 illustrates the data used in this modified model. Again, there was no statistically significant main effect of trial block,  $F(3, 51) = 1.84, p = .077, \omega^2 = 0.10$ , and no significant trial block x group interaction,  $F(3, 51) = 1.24, p = .152$ . There was, however, a main effect of group,  $F(1, 17) = 3.94, p = .032, \omega^2 = 0.19$ . These results suggest that heavily exposed syndromal boys took significantly longer path lengths to the invisible target than their non-exposed counterparts, although neither group improved their performance across trial blocks.



*Figure 8.* Mean path length for heavily-exposed syndromal and non-exposed boys across invisible trial blocks

In summary, the results of the place learning phase indicate that, as expected, boys performed better than girls on a task of map-based spatial navigation. Within the male participants, heavily-exposed syndromal boys performed significantly more poorly than non-exposed boys; non-exposed boys consistently located the target more efficiently. The efficiency of nonsyndromal boys to locate the target fell somewhere between that of syndromal and non-exposed boys. Amongst the female participants, both the exposed and non-exposed participants performed equally poorly and consistently took longer path lengths to locate the target than their male counterparts.

### **Phase III: Probe Trial**

The 16 invisible trials were followed by one probe trial, where the target was removed from the arena. Because the target had been in the NW quadrant for the duration of the place learning phase, the amount of time spent in the NW quadrant on the probe trial was recorded as a measure of cognitive mapping: the expectation here is that the more time the participant spent in the target quadrant on the probe trial, the more successfully he/she has mapped the environment (Morris, 1981; Jacobs et al., 1997). Figure 9 illustrates the proportion of the total probe trial time exposed and non-exposed participants spent in each quadrant.



Figure 9. Proportion of time spent in each quadrant on the probe trial.

Because time spent in the NW quadrant on the probe trial correlated significantly with mean path length on the invisible trials,  $r = -0.70$ ,  $p < .001$ , similar between-group differences as documented above were expected when analyzing probe trial performance. Univariate ANOVA analyses did not bear out those expectations, however. For instance, with regard to exposed versus non-exposed boys, there were no statistically significant between-groups differences,  $F(1, 25) = 0.17$ ,  $p = .341$ , suggesting that the boys spent a similar amount of time searching the NW quadrant, regardless of alcohol exposure.

Similar non-significant results were found for exposed versus non-exposed girls,  $F(1, 17) = 1.19$ ,  $p = .146$  and for all boys versus all girls,  $F(1, 44) = 1.32$ ,  $p = .129$ . Figure 10 shows the data used in these analyses.

Contrary to predictions, these results suggest that boys and girls performed equally in the probe trial, regardless of exposure to alcohol. They also suggest that the place learning advantage evident in males in the invisible trials did not translate into superior probe trial performance amongst the boys. This apparent discrepancy in my findings will be explained in the Discussion.

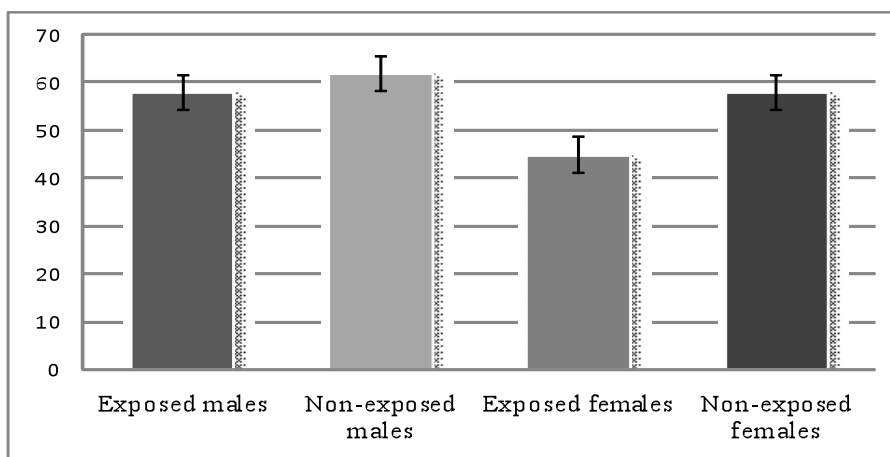


Figure 10. Time spent in the NW quadrant on the probe trial.

### Post-Experiment Questionnaire

This questionnaire was administered to obtain a self-report measure of the participants' search strategies, their previous gaming experience, and the perceived difficulty of the task. Table 3 presents the descriptive statistics for the questionnaire. During the place learning phase, it was expected that the participants would use the distal cues to aid them in locating the target. The table suggests that the number of children who used the cues did not differ significantly between the exposed and non-exposed participants. Similarly, the use of distal cues did not correlate significantly with the mean invisible trial path length,  $r = -0.07$ ,  $p = .667$ . The distribution of boys and girls who used distal cues to locate the invisible target fell just short of conventional levels of significance,  $\chi^2(1, 43) = 3.59$ ,  $p = .058$  with the proportion of boys (85%) who used the cues being higher than that of the girls (59%). The use of distal cues remained a non-significant correlate of invisible trial performance when split by sex, however,  $r = -0.16$ ,  $p = .424$ , and  $r = -0.09$ ,  $p = .723$ , for boys and girls respectively.

Table 3.  
*Descriptive Statistics for Post-Experiment Questionnaire*

Measure	Exposed ( $n = 29$ )	Non-exposed ( $n = 17$ )	Test statistic	$p$
Used distal cues to locate target <sup>a</sup>	20 (77%)	12 (71%)	0.22	.642
Have gaming experience <sup>a</sup>	14 (54%)	13 (77%)	2.25	.133
Hours of gaming per week <sup>b, c</sup>	2.04 (2.68)	5.41 (6.95)	-1.91	.071
Perceived difficulty	1.85 (0.78)	1.65 (0.79)	0.81	.421

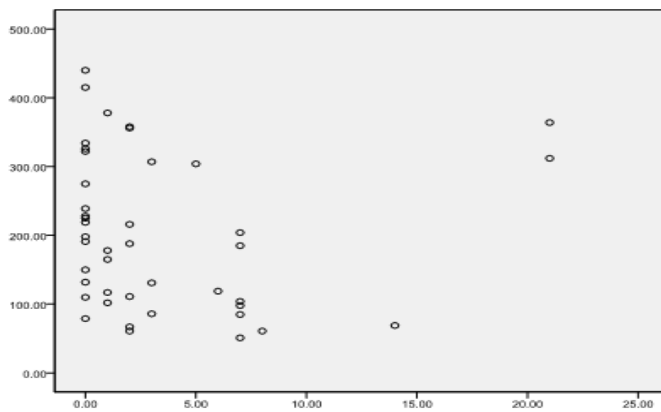
*Note.* The test statistic was either  $t$  or  $\chi^2$ . Data were missing for 3 children.

<sup>a</sup>Values reported are frequencies (%). <sup>b</sup>Values reported are mean (SD). <sup>c</sup>Game players only.

The proportion of children who had gaming experience did not differ between the exposed and non-exposed groups. Similarly, there were no statistically difference between-group differences for boys and girls in terms of gaming experience,  $\chi^2(1, 43) = 0.19$ ,  $p = .663$ . This latter result suggests that the sex differences reported earlier cannot be attributed to differences in gaming experience. This conclusion is further strengthened by the fact that gaming experience did not correlate significantly with mean invisible trial path length,  $r = 0.29$ ,  $p = .058$ . Although the correlation tended towards significance, the positive direction of the correlation rules out the suggestion that gaming experience enhances performance on tests of spatial navigation.



Each participant who reported having had prior gaming experience was asked to estimate the number of hours per week spent playing computer games. On average, the exposed children in this sub-group of participants played 2 hours of computer games per week, as opposed to an average of almost 6 hours per week in game-experienced non-exposed participants. This difference was found not statistically significant, however,  $t(19,14) = -1.91, p = .071$ . Similarly, there were no statistically significant male-female differences on this measure,  $t(41) = 0.88, p = .382$  (boys:  $M = 3.92, SD = 5.00$ ; girls:  $M = 2.53, SD = 5.14$ ). Overall, there was a non-significant negative correlation between the number of gaming hours per week and mean path length across the set of invisible target trials,  $r = -0.04, p = .785$ . Figure 11 illustrates this weak association.



*Figure 11.* Scatterplot illustrating correlation between hours of gaming per week and mean path length across the set of invisible target trials.

With regard to ratings of the difficulty of the task, both exposed and non-exposed children reported finding the task to be quite easy. Similarly, boys and girls both rated the task as equally easy,  $t(41) = 0.41, p = .682$  (boys:  $M = 1.81, SD = 0.90$ ; girls:  $M = 1.71, SD = 0.59$ ).

In summary, the post-experiment questionnaire data illustrate two points. First, the differences in place learning performance between boys and girls cannot be attributed to differences in gaming experience or perceived difficulty of the task. Second, the differences in place learning performance between exposed and non-exposed boys cannot be attributed to these variables either.

## **Discussion**

### **Cued Navigation Performance**

The results from the four visible target trials indicate that cued navigation is not affected by alcohol exposure or by sex. These results are consistent with the findings of previous studies using desktop-based virtual navigation tasks (Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005; Hamilton et al., 2003; Newhouse et al., 2007; Thomas, Laurance, Nadel, & Jacobs, 2010), and, as in those studies, suggest that neither prenatal alcohol exposure nor sexual dimorphism affects the neural mechanisms needed for efficient cued navigation ability.

Furthermore, the fact that no between-group differences in cued navigation were found suggests that all participants understood the task equally well and were equally motivated to find the target. It also suggests that girls and boys, regardless of group, had no difficulties using the equipment. This suggestion is further reinforced by the fact that there were no between-group differences in gaming experience or perceived difficulty of the task.

Therefore, one might safely conclude that, because the participants performed equally well on the visible platform trials, motivation, gaming experience, and difficulty of the task cannot account for the between-group differences found on the invisible platform trials.

### **Place Learning Performance**

Consistent with reports of impaired navigation abilities amongst alcohol-exposed animals (D'Hooge & De Deyn, 2001; McAdam, Brien, Reynolds, & Dringenberg, 2008), the findings of the current study illustrate place learning deficits amongst alcohol-exposed children. Specifically, when compared to non-exposed boys, alcohol-exposed boys consistently performed more poorly on the set of invisible target trials. These results are consistent with previous findings (Hamilton et al., 2003). Researchers have suggested that successful spatial navigation requires the use of a cognitive map, which is constructed by representing environmental stimuli, such as distal cues, in relation to one another (O'Keefe & Nadel, 1978). It is therefore possible to conclude that the alcohol exposed boys had difficulty acquiring, remembering, or using a cognitive map of the CG Arena, compared to the non-exposed boys.

With regard to sex differences on invisible target trials, girls performed more poorly than the boys. This difference in place learning performance between boys and girls tended towards significance, but fell just short of conventional levels. These results are not entirely

consistent with previous findings suggesting that there are robust sex differences in spatial navigation abilities amongst healthy participants, with young females performing more poorly than young males on a VMWT (Driscoll et al., 2005; Mühl, Kabisch, & Griego, 2005; Newhouse et al., 2007). In the current study, the fact that there was no significant sex difference in place learning performance may have been due to the fact that the expected male advantage on the set of invisible target trials was not present amongst the alcohol exposed boys. Because the hippocampus is the primary neural substrate underlying spatial navigation (Astur et al., 2002; Berman & Hannigan, 2000; O'Keefe & Nadel, 1978), this reduced advantage amongst the exposed boys may therefore result from an increased sensitivity to hippocampal damage amongst males following prenatal alcohol exposure compared to girls. No studies to date have tested that hypothesis; neuroimaging studies following, for instance, the fMRI protocols described by Pine et al. (2002) and comparing alcohol-exposed boys and girls to non-exposed controls might shed some light on the issue.

An alternative explanation for the lack of a significant sex difference in place learning performance is that subtle differences in performance between the exposed and non-exposed girls may exist, however the particular CG Arena protocol used in this study is not sensitive enough to capture these difference. Furthermore, it is possible that path length may be too gross a measure to capture such differences, therefore alternative measures of place learning ability, such as time taken to locate the target, as used in previous studies of spatial navigation, (Skelton, Bukach, Laurance, Thomas, & Jacobs, 2000; Thomas et al., 2001; Thomas et al., 2010), may be more appropriate.

An implication of the non-significant difference in female performance is that cognitive mapping tasks, such as the CG Arena, cannot be used as detection tools for prenatal alcohol exposure. It has been suggested that differences in place learning performance may be beneficial in discriminating between alcohol exposed children, who lack the distinctive facial characteristics of FAS, and non-exposed children. The results of this study suggest that this is not the case amongst girls, as the results were unable to discriminate between the alcohol exposed and non-exposed participants.

When the group of alcohol-exposed boys were split according to diagnostic group, the results replicated the findings of Hamilton et al. (2003). Specifically, the combined group of FAS and PFAS boys consistently took longer paths to locate the hidden target than the group of non-exposed boys. These results suggest that heavy prenatal alcohol exposure results in poor cognitive mapping abilities in comparison to non-exposed boys, as a result of hippocampal damage. The current data also suggested that, compared to the heavily-exposed

syndromal and non-exposed boys, nonsyndromal HE boys displayed an intermediate level of performance on the invisible trials (i.e., their performance did not differ significantly from either of the other groups). This result precludes the use of cognitive mapping tasks as potential detection tools for prenatal alcohol exposure amongst males: such tasks are not able to discriminate between HE boys, who are heavily exposed yet lack facial characteristics of FASD, and non-exposed boys.

### **Probe Trial Performance**

Research suggests that participants who have learnt to successfully spatially navigate during the set of invisible target trials go on to spend the majority of their probe-trial time searching the quadrant where that target had previously been located (Morris, 1981; Astur et al., 2002; Thomas et al., 2001). Contrary to what was expected, there were no statistically significant between-group differences (in terms of either exposure or sex) on the probe trial. With regard to exposure, these results are not consistent with Hamilton et al. (2003), who found that FAS boys spent significantly less time in the target quadrant than non-exposed boys. With regard to sex, the current results are not consistent with several studies showing that females perform significantly more poorly on probe trial-type measures (e.g., Astur et al., 1998; Newhouse et al., 2007; Thomas et al., 2010).

It should be noted, however, that in my sample the mean invisible path length was strongly negatively correlated with time spent in the target quadrant during the probe trial. The results indicated that the boys and girls, in both the exposed and non-exposed groups, performed similarly, suggesting that the advantage in visual-spatial learning, which the non-exposed boys displayed during the invisible trials, no longer existed in the probe trial. These results may suggest that time spent in the NW quadrant was not an adequate measure of probe trial performance, as it was not sensitive enough to capture the boys' superior cognitive mapping abilities.

Despite there being no statistically significant difference in performance between the participants (in terms of exposure and sex), it is important to analyze the perseverance inherent in the participants' search strategies. In the current sample, both the exposed and non-exposed participants spent the largest portion of their time searching the NW quadrant. The same pattern was evident when split by sex. Because I did not analyse the search paths of the participants, however, I am unable to determine their search strategies. It is possible that the participants navigated directly to the NW quadrant and persisted in searching that area of the arena first, however, I am unable to confirm this. The participants' persistence in the NW

quadrant does, however, suggest that they had created cognitive maps, which were used to remember the location of the target and navigate to it. Furthermore, the results suggest that when the participants were unable to locate the target, their search strategies were modified.

In the current study, 2 minutes elapsed before the probe trial automatically terminated. Although 2 minutes is the typical allotted time in several other CG Arena studies (e.g., Skelton et al., 2000; Thomas et al., 2010), it is considerably longer than the Hamilton et al. (2003) study, in which the probe trial terminated after 45 seconds. It is therefore possible that the non-significant differences in performance between alcohol exposed and non-exposed children are due to the fact that the probe trial was too long. Had the probe trial been shorter, it is possible that more distinct patterns of performance may have been found, as it is clear that the participants knew which quadrant to navigate to, but changed their search strategies after searching consistently in the NW quadrant for a considerable amount of time.

### **Post-experiment Questionnaire**

The results of the post-experiment questionnaire indicated that all participants, regardless of levels of prenatal alcohol exposure and sex, had similar levels of gaming experience and perceived the task to be similarly difficult. Amongst those who played computer games weekly, there were no statistically significant between-sex or between-exposure group differences with regard to number of hours spent playing games. These results are consistent with findings from previous studies of virtual navigation tasks with children (Hamilton et al., 2003; Newhouse et al., 2007). It can therefore be concluded that gaming experience and perceptions of task difficulty cannot account for the between-group differences in place learning performance.

The results of the post-experiment questionnaire also suggest that no difference in the use of distal cues existed between the exposed and non-exposed boys, despite having significantly different place learning abilities. This is likely to be due to the fact that the children did not understand the question or were not able to articulate their search strategy clearly to the research assistant. In situations, such as this, where the results of self-report measures contradict those of standardised test measures, the latter are considered more reliable, therefore the results of the performance measures should be more heavily weighted.

### **Limitations and Directions for Future Research**

Two limitations of the current study have already been mentioned, both of which pertain to the version of the CG Arena task used in the current study. The first concerns the

sensitivity of the task: it is possible that differences in exposed versus non-exposed female performance were not detected because the task was not sensitive enough to identify differences on the bottom end of the performance scale. In future investigations, it would be beneficial if the task was adapted to detect not only the major differences in performance, but also the subtle ones. The second limitation concerns the length of the probe trial: It is possible that the trial was too long, and thus obscured potentially significant performance differences. In future investigations of FASD children, the CG Arena probe trial should be shortened to be in line with previous studies and should not exceed 60 seconds.

A major limitation of the current study relates to sample size. Although the overall *N* was considerably larger than that of Hamilton et al. (2003) and roughly equal to that of Newhouse et al. (2007), when split by diagnostic group, the cell sizes became too small to run analyses by diagnosis. This was particularly true for the FAS group, resulting in the need to combine the FAS and PFAS participants. Future investigations using considerably larger samples would prove useful in determining the true nature of spatial navigation differences across the FASD spectrum.

In addition to the abovementioned limitations, the current study results are limited by the fact that no covariates were taken into consideration when performing statistical analyses. As noted in the Methods section, the groups differed on several maternal characteristics, and when these variables were added to the ANOVA models, they eliminated all significant findings. For instance, with regard to exposure, there were statistically significant between-group differences in terms of maternal education and maternal non-verbal intelligence, but by adding those variables to the model, it was no longer clear whether they, or actual exposure levels, were responsible for performance differences. According to Jacobson and Jacobson (2005), when alcohol exposure and a potential confounding variable are both included in a multivariate analysis of effects on a cognitive or behavioural outcome, the variable which was measured with greater accuracy will account for more variance in the outcome variable. In analyses, such as those employed in the current study, there is a risk that the true effects of prenatal alcohol exposure on place learning ability will be understated, when analysed in conjunction with maternal variables, merely because alcohol consumption was measured less reliably. In such cases, researchers run the risk of a Type II error occurring. Because the current study was exploratory in nature, I decided to be less conservative and not include potential confounding variables. In doing so, I decreased the chance of a Type II error occurring, but increased the risk of a Type I error.

## Summary and Conclusion

The results of the current study suggest that, as hypothesised, alcohol-exposed children do not present with deficits in cued navigation, but that prenatal alcohol exposure does result in poor place learning abilities in boys (Berman & Hannigan, 2000; Manji et al., 2009; McAdam et al., 2008; Uecker & Nadel, 1996). Male performance was also found to be significantly more impaired amongst heavily exposed syndromal boys, but not amongst nonsyndromal boys. The current findings were therefore unable to contribute to the existing debate as to whether a dose-response relationship exists between alcohol exposure and spatial navigation abilities.

As hypothesised, females tended to perform more poorly on a test of cognitive map-based spatial navigation than males (Blanchard et al., 1987; Newhouse et al., 2007; Thomas et al., 2010). This sex difference was not, however, as robust as that found in studies using healthy participants only (Driscoll et al., 2005; Mühl, Kabisch, & Griego, 2005). These results therefore suggest that alcohol-exposed males may be more sensitive to hippocampal dysfunction than females.

Current knowledge of the effects of alcohol exposure on hippocampus development, in conjunction with animal and human studies of the effects of hippocampal damage on spatial navigation, have served to emphasise the importance of the hippocampus as a structure vital for place learning. Desktop computer versions of the Morris water maze have proven to be extremely beneficial in identifying the neural correlates of place learning in humans. Furthermore, such studies have provided evidence in support of cognitive mapping theories and the numerous conditions under which cognitive mapping abilities are compromised. Further research into spatial navigation deficits in alcohol-exposed children will provide valuable insights into the functional impairments of FASD as well as contribute to our current understandings of the neural circuitry responsible for place learning.

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## Appendix A

## Ethical Approval for 9-year Follow Up Study

**WAYNE STATE  
UNIVERSITY**

HUMAN INVESTIGATION COMMITTEE  
101 East Alexandrine Building  
Detroit, Michigan 48201  
Phone: (313) 577-1628  
FAX: (313) 993-7122  
<http://hic.wayne.edu>




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## NOTICE OF EXPEDITED CONTINUATION APPROVAL

**To:** Sandra Jacobson  
Psychiatry  
University Square Office Plaza

**From:** Ellen Barton, Ph.D. \_\_\_\_\_  
Chairperson, Behavioral Institutional Review Board (B3)

**Date:** October 13, 2009

**RE:** HIC #: 099504B3F  
Protocol Title: Neuroimaging Studies of FAS Children in South Africa  
Sponsor: ° NATIONAL INSTITUTES OF HEALTH  
Protocol #: 0504001735

**Expiration Date:** October 12, 2010

**Risk Level / Category:** 45 CFR 46.404 - Research not involving greater than minimal risk

Continuation for the above-referenced protocol and items listed below (if applicable) were APPROVED following Expedited Review by the Chairperson/designee of the Wayne State University Institutional Review Board (B3) for the period of **10/13/2009 through 10/12/2010**. This approval does not replace any departmental or other approvals that may be required.

- Closed to accrual (date of closure 7/30/09)

° Federal regulations require that all research be reviewed at least annually. You *may* receive a "Continuation Renewal Reminder" approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval **before** the expiration date. Data collected during a period of lapsed approval is unapproved research and can **never** be reported or published as research data.

° All changes or amendments to the above-referenced protocol require review and approval by the HIC **BEFORE** implementation.

° Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the HIC Policy (<http://www.hic.wayne.edu/hicpol.html>).

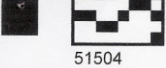
**NOTE:**

1. Upon notification of an impending regulatory site visit, hold notification, and/or external audit the HIC office must be contacted immediately.
2. Forms should be downloaded from the HIC website at *each* use.

\*Based on the Expedited Review List, revised November 1998

## Appendix B

### Post-experiment Questionnaire



51504

### Cape Town 9 year follow-up Virtual Water Maze

ID    Examiner    \ \_\_\_\_\_ Day   Month   Year

**EXAMINER OBSERVATIONS:**

Was a practice round used?.....  Yes  No

Did the child require further instructions?.....  Yes  No

Which hand did the subject use?.....  Right  Left  Both

Did you prompt the child about turning on the platform on trial 1?  Yes  No

Other prompts/feedback given: \_\_\_\_\_

\_\_\_\_\_

Attitude towards task...  Relaxed  Frustrated/agitated

Attentiveness:.....  Alert throughout  Became bored  Fatigued

Child's position:.....  Stays seated  Stands up  Combination

Strategy category:

Luck/impulsive (no recognizable strategy)

Deliberate/skilled  
(child recognizes general platform area and tried their best to target that area)

Other behavioral observations: \_\_\_\_\_

\_\_\_\_\_

**POST-TEST QUESTIONS:**

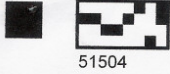
1. How did you find the platform? \_\_\_\_\_

Were there any parts of the game that helped you find the platform?.....  Yes  No

If YES, which? \_\_\_\_\_

Did you change how you found the platform from the first time to the last time?...  Yes  No

If YES, how? \_\_\_\_\_



### fMRI Study - Cape Town Virtual Water Maze - page 2

ID 

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2. Do you think the platform was always in the same place or did it move each time?  Same  Moved

If SAME, where do you think the platform was? \_\_\_\_\_

If MOVED, where do you think the platform usually was? \_\_\_\_\_

3. Do you think you started in the same place in the pool each time, or did you start at different places?  Same  Different

4. Do you play video or computer games?  Yes  No (skip to #5)

How many days per week do you play them?..... 

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 - 

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How many hours per day do you play for on days that you play them? 

--	--

 - 

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What are some of your favorite games right now? \_\_\_\_\_  
\_\_\_\_\_

5. Did you think this game was:

Very easy  Kind of easy  Sort of hard  Very hard

## Appendix C

### Consent Form

**Neural Bases of Eyeblink Conditioning in FASD  
Parental Permission/Research Informed Consent  
Title of Study: Neural Bases of Eyeblink Conditioning in FASD**

We are pleased to invite you and your child \_\_\_\_\_ to continue to take part in the study that you have been in since you were pregnant and your baby was born. Please read this form and ask us any questions you have before agreeing to be in the study. The people conducting this study are doctors and scientists from the Faculty of Health Sciences of the University of Cape Town School in South Africa and Wayne State University School of Medicine in the United States: Ernesta Meintjes, Ph.D., and Christopher Molteno, MD., from University of Cape Town, and Sandra W. Jacobson, Ph.D., and Joseph L. Jacobson, Ph.D., from Wayne State University in the United States. It is being paid for by the National Institute on Alcohol Abuse and Alcoholism in the United States and the Department of Science and Technology and the National Research Foundation of South Africa.

**Study Purpose:** In this study we want to learn whether some aspects of a child's thinking and behavior are different when a mother drinks or and smokes during pregnancy, and whether genes (characteristics that you inherit from your parents) make it more or less likely that the child will show these differences. Other purposes of the study are to see whether your child's abilities when s/he was a baby and 5 years old predict how he or she is doing 8-10 years of age. To help decide whether or not to agree to take part with your child in this study, a project staff member has talked with you about the risks and benefits of the study. This consent form summarizes the information given to you by the project staff member during this informed consent process.

The study will use new methods for studying the brain called MRI neuroimaging to better understand how drinking alcohol and smoking during pregnancy can affect a child's development. In neuroimaging, the child lies in a scanner that uses magnets to take pictures of the brain. In this part of the study, we will take pictures on the new scanner at Tygerberg Hospital while your child lies still and watches a video and does some simple finger tapping, attention, and memory tasks.

**Study Procedures:** If you agree to have your child take part in this study, we will bring you and your child to the our laboratory at the University of Cape Town (UCT) for 2-3 visits that will each take about 4 hours and to Tygerberg Hospital for one visit that should take about 3 4 hours in total.

Submission date: 01/09/08  
Protocol Version #: 1

Parent Initials \_\_\_\_\_

- During the visits to University of Cape Town, your child will do simple tasks involving finger tapping, attention, learning and memory, arithmetic, word meanings, puzzles, circle drawing, and mazes (Wechsler Intelligence Scale for Children; paced/unpaced finger tapping; Circle Drawing task; timing and pitch perception tasks; California Verbal Learning Test).
- We will test your child's vision.
- In one task, your child will put on a special helmet. While your child is watching a video, a puff of air from the helmet will cause him/her to blink while hearing a tone to see if s/he learns to use the tone as a signal to blink before the air puff arrives.
- We will weigh and measure your child and take a photograph to look for facial features that often relate to alcohol exposure during pregnancy.
- During this visit, we will ask you some questions about your child's behavior and attention (Disruptive Behavior Disorders assessment), daily activities (Child Behavior Checklist), school and health history, and any medications that s/he is taking.
- We will ask you to update us about stressful experiences in your daily life during the past year (Life Events Scale), your current drinking, smoking, and drug use, attention problems you may have had as a child (Barkley-Murphy ADHD Scale), and stressful feelings that you experience, including sadness, anxiety, and distress (Beck Depression Inventory; Structured Clinical Interview for DSM-IV).
- At the end of the first visit, our research driver and nurse will take you and your child to a nearby clinic, where a technician/nurse will take a 5 cc blood sample (approximately 1 teaspoon) from your child's vein to test for lead and iron deficiency anemia. About 10 cc of blood (about 2 teaspoons) will be obtained from your child and yourself to study genetic differences that you and your child inherited from your family and have been found to be related to differences in alcohol use, depression, attachment, or child attention/behavior and development. We will also ask you and your child to give a small sample of saliva (about 1 teaspoon) to study genetic differences that have been found to be related to differences in alcohol metabolism, depression, attachment or child attention/behavior, and development. These samples will be stored and used for future genetic analyses.
- During the visit to Tygerberg, your child will first practice the finger tapping, and attention and memory tasks s/he will be doing on a computer while lying in the scanner. During the neuroimaging, your child will lie on a padded plastic bed that slides into the scanner. We will ask him/her to lie as still as possible while the pictures are being taken. Taking these pictures of the brain does not hurt and is used every day by many people in the hospital. During some of the time in the scanner, your child will watch videos and during some of the time s/he will do the finger tapping and other tasks that were practiced before entering the scanner. There will be two sessions in the scanner-both on the same day-one in the morning and one after lunch, which we will give you and your child while you are at Tygerberg. Each session in the scanner will last no longer than 45-60 minutes. Children with the following may not have an MRI but will take part in the rest of the visits: implanted medical devices, such as aneurysm clips in the brain, heart pacemakers, and cochlear (inner ear) implants; lead-based tattoos; or pieces of metal close to or in an important organ (such as, the eye); claustrophobia or fear of being in a small space.

Submission date: 01/09/08

Parent Initials\_\_\_\_\_



Protocol Version #: 1

**Benefits:** There may be no direct benefits for you; however, information from this study may help other people now or in the future. We will give you information about your child's development at this age. We will use the findings from this study for research purposes only. However, if a serious problem is found, we will tell you and refer your child to a doctor and/or someone who can help, if you would like us to do so. If your child is suffering from any major illness, we will send you Red Cross Children's Hospital. No information about your child will be given to any doctors, hospitals, or schools unless you ask us and allow us to do so in writing.

**Risks:** None of the procedures we use at UCT or Tygerberg are dangerous for you or your child. The risks of drawing blood include some temporary discomfort or swelling, and rarely, infection. These risks will be minimized because the procedure will be done by a trained phlebotomist (nurse/technician who has been specially trained to draw blood). We will begin by introducing you and your child to the research staff and will give you both breakfast each day before the assessment begins. You will be present in a room nearby during all of your child's assessments and will be present with your child during the physical examination and blood draw. During the MRI neuroimaging assessment, certain metal objects, such as, watches, credit cards, hairpins, and writing pens, may be damaged by the MRI scanner or pulled away from the body by the magnet. For these reasons, we will ask your child to remove these before going into the scanner. When the scanner makes the pictures, the bed may shake, and your child will hear loud banging noises. S/he will be given earplugs or headphones to protect the ears. Also, some people feel nervous in a small closed space, such as when they are in the scanner. Your child will be able to see out of the scanner at all times, and we will not start until s/he tells us that s/he is comfortable. S/he will be able to stop the scanning at any time by squeezing a ball that s/he will hold in one hand and can talk to us using an intercom that is built into the scanner. There are no known harmful long-term effects of the magnetic fields used in this study. (There is little risk that anything we tell you will be told to people outside the study and we will do everything we can to keep this information secret, as described below, except that evidence of child abuse or neglect will be reported to the appropriate authorities, as required by law, and may report other illegal activities that are reported to us during the visit.

**Research Related Injuries:** If you or your child is injured during the study, you will get treatment including first aid, emergency treatment and follow-up care, as needed. No reimbursement, compensation, or free medical care is offered by Wayne State University or the University of Cape Town. If you think that your child has suffered a research related injury, let the investigator know right away.

Submission date: 01/09/08

Parent Initials\_\_\_\_\_

Protocol Version #: 1

**Study Costs:** There will be no cost to you or your child for taking part in this research study, and you and your child will be transported to the laboratory at University of Cape Town and Tygerberg Hospital by our driver.

**Compensation:** For taking part in this research study, we will give you R150 (\$25) for each visit and a photo of your child, and we will give your child a small gift. You and your child will also be given breakfast and lunch each time you and your child come to University of Cape Town or Tygerberg Hospital.

**Confidentiality:** We will keep all information collected about you and your child during the study secret to the extent permitted by law. This information will not be used in any way that can allow anyone else to know what you or your child has told us, except that evidence of child abuse or neglect will be reported to the appropriate authorities, as required by law. You and your child's names will not be in the research records, only your code number. We will not give out any information that names you or your child unless you give us written permission, but your records may be reviewed by the study sponsor, the Human Investigation Committee at Wayne State University, the University of Cape Town Research Ethics Committee, or governmental agencies with appropriate regulatory oversight. The list linking names and code numbers will be stored in locked file cabinets in the research laboratory. Only project staff members who need to contact you by telephone or in person will be allowed to look in these files. Information from this study, including photos may be presented in scientific meetings or journals or for teaching purposes, but your and your child's names will be kept secret.

**Voluntary Participation/ Withdrawal:** Taking part in this study is voluntary. You may decide to have your child take part and later change your mind and quit the study. You and your child are also free not to answer any questions or to stop any task before it is finished. Withdrawal from the study would not lead to any problems for you or your child. The researcher or the sponsor may also stop your child's taking part in this study without your agreeing to it.

**Questions:** If you have any questions now or in the future, you may contact Drs. Ernesta Meintjes or Christopher Molteno at 021-406-6212 or Dr. Sandra W. Jacobson at 001-313-9935454. If you have questions or concerns about you or your child's rights as a research participant, you can contact the Chairs of either the University of Cape Town Research Ethics Committee (021 406-6338) or the Wayne State University Human Investigation Committee (001-313-577-1628).

Submission date: 01/09/08  
Protocol Version #: 1

Parent Initials \_\_\_\_\_

**Consent to Participate in a Research Study:** To voluntarily agree to have your child take part in this study, you must sign on the line below. If you decide to take part with your child, you or your child may quit at any time. You are not giving up any of your or your child's legal rights by signing this form. Your signature shows that you have read, or had read to you, this whole consent form, including the risks and benefits, and that we have answered all your questions. We will give you a copy of this consent form to take home.

\_\_\_\_\_  
Signature of Parent or Legally Authorized  
Guardian

\_\_\_\_\_  
Date

\_\_\_\_\_  
Printed Name of Parent or Authorized  
Guardian

\_\_\_\_\_  
Time

\_\_\_\_\_  
Oral Assent (children age 7-12 years)

\_\_\_\_\_  
Date

\_\_\_\_\_  
\*\*Signature of Witness (When applicable)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Printed Name of Witness

\_\_\_\_\_  
Time

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Date

\_\_\_\_\_  
Printed Name of Person Obtaining Consent

\_\_\_\_\_  
Time

\*\*Use when parent has had consent form read to them (i.e. illiterate, legally blind, translated into foreign language).

## Appendix D

### CG Arena Descriptive Statistics for Visible and Invisible Platform Trials

Trials	Alcohol exposed				Non-exposed			
	Boys ( <i>n</i> = 19)		Girls ( <i>n</i> = 10)		Boys ( <i>n</i> = 8)		Girls ( <i>n</i> = 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Visible trials								
V1	62.3	26.8	90.1	72.6	56.5	9.2	51.2	22.1
V2	35.4	25.9	43.3	41.1	25.9	5.2	26.5	16.9
V3	67.8	33.5	77.9	32.0	61.4	21.2	47.7	18.2
V4	28.4	13.3	40.2	40.6	20.9	2.1	22.6	9.8
Invisible trials								
I1	331.5	217.1	252.9	182.7	198.5	99.0	160.3	107.2
I2	213.4	187.5	242.9	170.6	135.8	175.3	194.0	103.2
I3	185.6	191.6	135.3	165.6	80.0	84.5	223.0	219.4
I4	148.5	186.1	321.4	288.3	84.6	91.3	163.2	203.1
I5	299.3	289.2	187.2	195.4	179.2	154.6	328.5	236.3
I6	122.6	120.7	219.1	246.0	173.7	180.5	229.5	198.3
I7	246.3	270.5	261.1	196.4	109.2	63.7	262.4	229.7
I8	223.3	250.9	159.7	143.4	179.2	255.6	181.6	159.1
I9	251.3	261.5	238.8	290.1	123.3	134.4	262.5	227.7
I10	291.5	254.3	329.7	299.5	79.7	72.3	152.5	103.8
I11	178.7	176.7	309.4	255.5	123.9	171.6	151.9	102.3
I12	237.9	115.4	302.7	269.9	112.2	101.1	231.7	200.2
I13	161.4	195.5	250.2	244.0	66.9	46.2	256.6	177.9
I14	150.5	213.6	312.8	293.1	64.8	58.1	313.9	277.4
I15	170.5	202.3	260.7	266.3	171.1	245.0	195.5	285.6
I16	214.6	205.0	340.3	251.9	144.5	103.2	256.1	240.5

